JOURNAL OF THE SOUTH AFRICAN CHEMICAL INSTITUTE

NEW SERIES
VOLUME V
NUMBER ... I

JUNE
1952
JUNIE

Published by the Institute, Kelvin House,
Uitgegee deur die Instituut, Kelvinhuis,
JOHANNESBURG

Price 7/6
Prys 7/6

NUWE SERIE
DEEL V
NOMMER ... I

JOERNAAL VAN DIE SUID-AFRIKAANSE CHEMIESE INSTITUUT

CONTENTS—INHOUD

STUDIES OF THE FATS FROM INDIGENOUS SOUTH AFRICAN PLANTS

I. THE SEED FAT OF ACACIA GIRAFFAE (KAMEELDOORN)

by

G. S. HARRISON and F. HAWKE

OPSOMMING

'n Monster van die saad vet van *Acacia giraffae*, is geëkstraheer en die fisiese en chemiese konstante bepaal asook die samestellings van die vetsure en die onverseepbare materiaal. Die sade bevat omtrent 3·5 persent vet, wat die volgende konstante toon:

S.G. $d^{40°}_{4°} = 0{\cdot}9104$; $[n]^{40°}_{D} = 1{\cdot}4683$; $\sqrt{} = 38{\cdot}55$ c.s. teen 40° C.

Suurgetal = 3·69; versepingsgetal = 184·8; joodgetal = 112·9; hidroxielgetal = 19·6; onverseepbare materiaal = 4·48 persent; Reichert-Meisel getal = 0·1; Polenske getal = 0·5. Die vet lewer 90·4 persent vetsure en 9·76 persent gliserien en toon die volgende vetsuursamestelling (bereken as gewig persent): miristiensuur = spoor; palmitiensuur = 12·8; steariensuur = 5·6; arachiensuur = 1·7; behensuur = 0·9; tetradeceniensuur = spoor; palmitoliensuur = 7·3; oleinsuur = 23·5; eicosenien-suur = 1·4; hexadecadieniensuur = 1·1; linolsuur = 41·5; linoleensuur = 4·2.

SUMMARY

A sample of the seed fat of *Acacia giraffae* was extracted and the physical and chemical constants determined, together with the fatty acid composition and the composition of the unsaponifiable matter. The seeds contain about 3·5 per cent of fat having the following constants:

Sp. gr. $d^{40°}_{4°} = 0{\cdot}9104$; $[n]^{40°}_{D} = 1{\cdot}4683$; $\sqrt{} = 38{\cdot}55$ centistokes at 40° C.

Acid val. = 3·69; sap. val. = 184·8; I.V. = 112·9; hydroxyl value = 19·6; unsap. = 4·48 per cent; R.M. val. = 0·1; Polenske val. = 0·5. The fat yields 90·4 per cent of fatty acids and 9·76 per cent of glycerine and has the following fatty acid composition (weights per cent): myristic acid = trace; palmitic acid = 12·8; stearic acid = 5·6; arachidic = 1·7; behenic = 0·9; tetradecenoic = trace; palmitoleic 7·3; oleic = 23·5; eicosenoic = 1·4; hexadecadienoic = 1·1; linoleic = 41·5; linolenic = 4·2.

The Kameeldoorn (*Acacia giraffae*) is found in the sandy regions in the Northern Cape Province, the sandy basaltic loams around Potgietersrust in the Transvaal and also in the Bloemhof district, Bechuanaland and South West Africa. It is a tree from 25–40 ft. high with spreading branches which form an umbrella-shaped crown and which are covered with stout brown thorns. The plant bears large, somewhat curved, pods averaging 4 in. long by 1½ in. broad. These are covered with a dense grey felt and have thick spongy walls.

The average weight of a seed pod is 15 g. and there are usually between 18 and 20 seeds in a pod forming about 35 per cent of the total weight. The seeds themselves are tough and very hard with an average weight of 270 mg.

At present the only use for the pods is as a cattle feed. When ground to a fine meal, they are very palatable to cattle and are reported to increase the milk yield.[1]

Extraction of the fat

The seeds were separated from the pod husks by pounding the whole pods and sorting out the seeds by hand. These were ground to a fine meal in a laboratory hammer mill after preliminary crushing in cracking rolls. This meal was then extracted in a specially designed stainless-steel extractor using Shell "Special Boiling Petroleum Benzene" (Boiling Range 50°–70° C.). The extractor operated on the Soxhlet principle

but the vapours passed up through a central tube in the extractor body, thus conferring the added advantage of extraction with hot solvent. "Special Boiling Petroleum Benzene" was used in preference to Petroleum ether, because, while being considerably cheaper than the latter, it was found to be in no way inferior.

General characteristics of the fat

The fat is a bright orange-coloured liquid at 40° C. but on cooling to 25° C. solid matter separates out. This was at first considered to be due to high-melting glycerides but was later shown to be due to the presence of phosphatides and a small quantity of a wax from the testa. Similar observations have been reported in the case of Soya bean oil,[2] which this fat was found to resemble in many ways.

Filtration of the fat removed the solid temporarily, but, on standing, a solid phase was found to separate again. Preliminary extraction of the whole (uncrushed) seeds was found to remove part but not all of the solids. The complete removal was eventually effected by filtering the fat and washing the filtered fat with a boiling brine solution to emove phosphatides and mucilaginous matter.

Physical constants

These were determined on the freshly extracted fat, without further treatment, at the temperature at which it was completely homogeneous, viz.: 40° C.

Specific gravity $d^{40°}_{4°}$ = 0·9104

Refractive index $[n]^{40°}_{D}$ = 1·4683

$[n]^{45°}_{D}$ =1·4665

$[n]^{50°}_{D}$ = 1·4647

Temperature coefficient of refractive index = — 0·00036 per ° C.
Optical activity negligible
Surface tension = 32·91 dynes/cm. at 40° C.
Interfacial tension with water = 0·42 dynes/cm. at 40° C.
Viscosity √ = 38·55 centistokes at 40° C.
μ = 36·14 centipoises at 40° C.

The temperature coefficient of the refractive index corresponds to that satisfying the general equation for fats at temperatures near 40° C.[3]

The extremely low interfacial tension against water explains the ease with which the fat formed stable emulsions in all proportions. These emulsions were stable to prolonged centrifugation and also to the action of electrolytes. However, they were broken by the addition of controlled quantities of ethyl alcohol.

This property was shown to be due to the presence of phosphatides and mucilaginous material in the fat. When these were removed by washing the fat with boiling brine solution, the fat was no longer emulsified on shaking with water and the interfacial tension was increased to 20·0 dynes/cm. at 40° C.

Chemical constants

Acid value	= 3·69 mg. KOH/g.
Saponification value	= 184·8 mg. KOH/g.
Iodine value (Wijs)	= 112·9 g. I_2/100 g.
Iodine value (bromine vapour)	= 113·0 g. I_2/100 g.
Hydroxyl value	= 19·60 mg. KOH/g.
Unsaponifiable matter	= 4·48 per cent
Reichert Meisel value	= 0·1
Polenske value	= 0·5
Fatty acids	= 90·4 per cent
Glycerine	= 9·76 per cent
Mean molecular weight of fatty acids	= 284·3 (by direct titration)

The negligible difference between the Wijs iodine value and that determined by the bromine vapour method indicates the absence of conjugated double bonds in the fat.

The mean molecular weight of the fatty acids was calculated from the formula derived by Schoeman[4] and found to be 278·1. The discrepancy between this calculated value and that determined on the mixed fatty acids was explained in the light of further investigations and is discussed below.

The glycerine content is very close to that calculated from the formula[4]:—

$$G = 0 \cdot 05467\ E \text{ which is } 9 \cdot 75 \text{ per cent}$$

Fatty acid composition

160 g. of fat was saponified and the unsaponifiable matter extracted with ether. The soaps were then split and the fatty acids extracted with ether. This yielded 142·6 g. of fatty acids which were then dissolved in acetone for preliminary separation by low-temperature crystallization.

A total of 4,420 ml. of dry acetone was required to dissolve all the acids at room temperature. The solution of fatty acids was cooled to — 30° C. and allowed to crystallize at this temperature for six hours when 229·6 g. solvated fatty acids were filtered off. This yielded 32·7 g. of solid fatty acids (AG/A/30) which were then esterified with absolute anhydrous ethanol and fractioned in an "E.H.P." column.[5]

The filtrate from the above crystallization was further crystallized at — 60° C. for six hours, after which 173·8 g. of solvated fatty acids were filtered off. This yielded 49·0 g. of liquid fatty acids (AG/A/60) which were esterified with anhydrous methanol and fractionated. The filtrate from this latter crystallization yielded 59·0 g. of fatty acids (AG/A/60F) which were also esterified with anhydrous methanol and fractionally distilled.

The following tables give the results of the ester fractionations:—

AG/A/30

Weight of esters distilled	= 29·529 g.
Saponification equivalent	= 305·2
Saponification value	= 183·8
Iodine value	= 31·27
Hydroxyl value	= 2·89

Sample	*Weight (g.)*	*Sap. equiv.*	*Sap. val.*	*Iod. val.*	*Hydroxl val.*
I	1·694	283·3	197·9	7·30	—
II	1·955	293·2	191·3	25·08	—
III	3·082	294·7	190·3	25·21	—
IV	5·142	296·3	189·4	28·27	—
V	2·744	300·0	187·0	36·21	—
VI	1·884	299·7	187·2	37·02	—
VII	1·113	293·2	191·4	37·73	—
VIII	1·385	296·2	189·4	41·83	2·01
IX	2·200	304·1	184·5	47·18	—
X	1·791	307·5	182·0	45·88	—
XI	1·993	326·3	172·0	38·31	—
XII	0·739	346·3	161·9	19·90	—
XIII	3·253	366·3	154·1	14·48	18·97
Total	28·975				

To check on the constants of the samples, the total saponification and unsaturation products were divided by the sum of the sample weights to give the calculated values of the constants of the mixed esters.

The results were as follows:—

	Direct determination	*Calculated*
Sap. val.	183·8	183·9
Iod. val.	31·27	30·29
Hodroxyl val.	2·89	2·29

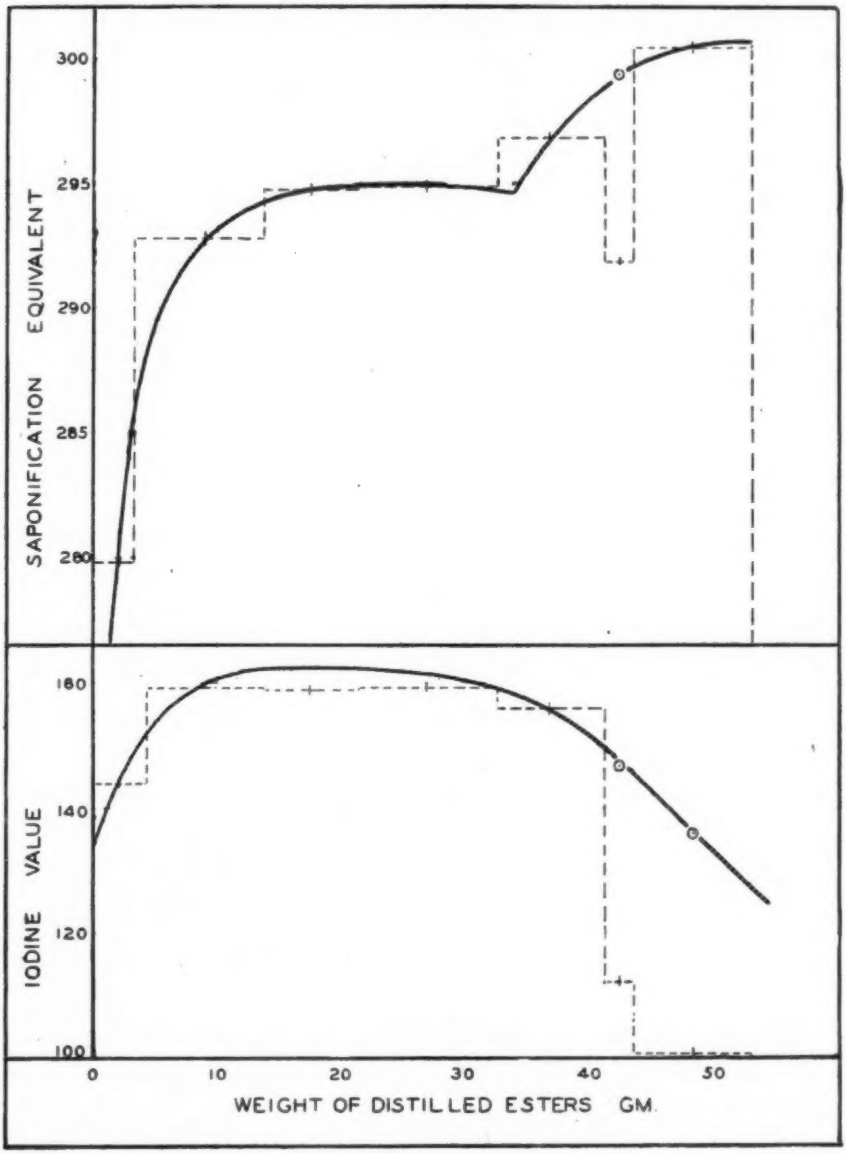
It will be noticed that the calculated saponification value is very close to that of the original crystal fraction, as is also the hydroxyl value. The calculated iodine value, however, is one unit lower than that of the crystal fraction.

This was probably due to the oxidation of the column and flask residues as shown by the abrupt fall in iodine values of these fractions (XII and XIII) as compared with those of the preceeding ones. Hilditch[6] recommends that the iodine and saponification values of the residues be assumed equal to those of the last distilled fraction, but this gives a degree of unsaturation higher than that of the total crystal fraction.

It was found that when values of iodine value versus weight of esters distilled and saponification equivalent versus weight of esters distilled, were plotted, they fell on smooth curves except for the iodine values of the two residual fractions. If, however, the curves were produced and values extrapolated for these iodine values, a degree of unsaturation much closer to that of the original crystal fraction was obtained. Consequently, these extrapolated iodine values, and, in later determinations, extrapolated saponification equivalents as well, were used in preference to the determined values or those based on Hilditch's assumption.

This method is indicated in Fig. 1, which is a graph of the values determined on AG/A/60F.

The iodine values for fractions XII and XIII were corrected to 32·1 and 22·0 respectively giving a calculated value of 31·34 as compared with 31·27 determined on the original sample AG/A/30.



300
295
290
285
280
SAPONIFICATION EQUIVALENT
160
140
120
100
IODINE VALUE
0
10
20
30
40
50
WEIGHT OF DISTILLED ESTERS GM.

FIG. I.

AG/A/60

Weight of esters distilled = 44.840 g.
Saponification equivalent = 291·2
Saponification value = 192·7
Iodine value = 117·1
Hydroxyl value = 3·26
Thiocyanogen value = 77·8

Sample	*Weight g.*	*Sap. equiv.*	*Sap. val.*	*Iod. val.*	*Hydroxyl val.*
XIV	7·582	288·6	194·4	108·2	—
XV	3·930	292·5	191·8	117·5	—
XVI	2·215	290·3	193·2	112·0	—
XVII	6·515	291·6	192·4	117·6	—
XVIII	5·096	289·7	193·6	120·1	—
XIX	10·029	290·3	193·3	118·4	—
XX	4·159	292·6	191·7	113·9	—
XXI	1·107	273·8	204·9	73·9	25·04
XXII	3·868	294·4	190·6	83·8	30·20
Total	44·501				

The saponification, iodine and hydroxyl values of the mixed esters were calculated as for AG/A/30.

As in the previous fraction, there is a sudden drop in the iodine values of the column and flask residues, and also in the saponification equivalent of the column fraction. These were corrected by extrapolating values on curves as described above.

The iodine values of fractions XXI and XXII became 113·6 and 112·2 respectively while the saponification equivalent and saponification value of fraction XXI became 293·5 and 191·2 respectively.

The following are the calculated values for the esters before and after correcting for oxidation.

	Direct determination	*Calculated*	
		Before allowing for oxidation	*after allowing for oxidation*
Sap. val.	192·7	193·1	192·8
Iod. val.	117·1	114·1	116·9

AG/A/60F

Weight of esters distilled = 53·181 g.
Saponification equivalent = 295·2
Saponification value = 190·0
Iodine value = 152·4
Thiocyanogen value = 96·92
Hydroxyl value = 27·49

Sample	*Weight (g.)*	*Sap. equiv.*	*Sap. val.*	*Iod. val.*	*CNS val.*	*Hydroxyl val.*
XXIII	4·256	279·8	200·5	143·9	93·03	8·28
XXIV	9·609	292·7	191·7	159·1	90·72	3·47
XXV	7·421	294·7	190·4	158·8	92·32	3·88
XXVI	11·288	294·8	190·3	159·1	94·77	3·41
XXVII	8·539	296·8	189·1	156·0	110·50	4·82
XXVIII	2·511	291·8	192·3	112·3	101·20	45·90
XXIX	9·415	300·4	186·7	100·6	97·31	59·93
Total	53·039					

The saponificiation, iodine and thiocyanogen values of the mixed esters were calculated as above.

The effects of oxidation on the column and flask residues were allowed for as shown in Fig. 1 and the saponification, iodine and thiocyanogen values of the mixed esters recalculated.

The results are tabluated below.

	Direct determination	*Calculated*	
		Before allowing for oxidation	*after allowing for oxidation*
Saponification val....	190·0	190·3	190·0
Io l. val.	152·4	144·2	152·6
CNS. val.	96·9	96·7	97·1

After the presence of hydroxylated compounds in the fatty acid and ester fractions was proved by means of hydroxyl values, these were calculated in terms of hydroxy fatty acids.

In an attempt to isolate and identify these acids, the mixed fatty acids from 400 g. of fat were crystallized from acetone at — 78 °C. at a concentration of 1:25. The hydroxy-acids were expected in the filtrate but instead a white solid was observed to separate out on concentrating the filtrate to about 1:3. This was filtered off and found to have the following properties.

Hydroxyl value = 368·1 mg. KOH/g.
Iodine value = 103·7 g. I_2/100 g.
Acid value = 16·3 mg. KOH/g.

The acid value was calculated to linolenic acid and the iodine and hydroxyl values corrected accordingly, giving the "non-saponifiable" matter an iodine value of 89·1 and a hydroxyl value of 389·7.

That there was still some hydroxy body remaining in the crystallized fraction (at — 78° C.) and the filtrate left after the removal of the solid material was shown by hydroxyl values of 11·0 and 23·1 respectively.

All attempts at complete removal of the hydroxy body from the fatty acids were unsuccessful and the fatty acid composition, therefore, was recalculated from the above data assuming the hydroxyl value to be due entirely to an hydroxylated substance of OH value 390 and iodine value 89.

The above "non-saponifiable" matter was analysed for elements by the Lassaigne sodium fusion and found to contain nitrogen. This together with the above characteristics and its insolubility in alcohol, ether, acetone, chloroform and water would seem to indicate that the "non-saponifiable" matter was sphingosin.

This has an hydroxyl value of 387·1 and an iodine value of 84·9 which are within the limits of accuracy of the determination carried out on the "non-saponifiable" matter where only very small quantities were available. (The yield from 400 g. of fat was 0·7g.)

Composition of ester fractions

AG/A/30

Fract.	*Wt.* (*g.*)	*Saturated esters*					*Mono-ethenoid esters*				"*Non-sap.*"
		C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{14}	C_{16}	C_{18}	C_{20}	
I ...	1·694	0·058	1·499	—	—	—	0·005	0·132	—	—	—
II ...	1·955	—	0·928	0·460	—	—	—	0·337	0·230	—	—
III ...	3·082	—	1·338	0·842	—	—	—	0·487	0·415	—	—
IV ...	5·142	—	1·915	1·530	—	—	—	0·820	0·877	—	—
V ...	2·744	—	0·665	0·905	—	—	—	0·414	0·760	—	—
VI ...	1·884	—	0·480	0·626	—	—	—	0·335	0·443	—	—
VII ...	1·113	—	0·523	0·259	—	—	—	0·134	0·197	—	—
VIII ...	1·385	—	0·396	0·313	—	—	—	0·329	0·347	—	—
IX ...	2·200	—	0·293	0·668	—	—	—	0·290	0·944	—	0·005
X ...	1·791	—	0·132	0·663	—	—	—	0·098	0·898	—	—
XI ...	1·993	—	—	0·492	0·519	—	—	—	0·408	0·574	—
XII ...	0·739	—	—	—	0·233	0·190	—	—	—	0·316	—
XIII ...	3·253	—	—	—	1·318	0·938	—	—	—	0·835	0·162
Total...	28·975	0·058	8·169	6·758	2·070	1·128	0·005	3·376	5·519	1·725	0·167

AG/A/60

Fraction	*Wt.* (*g.*)	*Saturated esters.*		*Mono-ethenoid esters.*		*Di-ethenoid esters*	"*Non-sap*"
		C_{16}	C_{18}	C_{16}	C_{18}	C_{18}	
XIV	7·582	1·151	—	0·717	2·678	3·036	—
XV	3·930	0·424	—	—	1·643	1·863	—
XVI	2·215	0·413	—	—	0·721	1·081	—
XVII	6·515	0·925	—	—	1·357	4·233	—
XVIII	5·096	1·024	—	—	1·037	3·035	—
XIX	10·029	1·824	—	—	2·618	5·587	—
XX	4·159	0·446	—	—	1·918	1·795	—
XXI	1·107	0·300	0·054	—	—	0·688	0·065
XXII	3·868	1·240	—	—	—	2·320	0·308
Total	44·501	7·747	0·054	0·717	11·972	23·638	0·373

The presence of 0·85 mg. phosphorus per g. of seed fat is indicative of the presence of phosphatides which usually include lecithins, cephalins and sphingomyelins. The removal of these by treating the fat with a boiling solution of sodium chloride, resulted in the removal of hydroxy bodies from the fatty acids which then had zero hydroxyl value, indicating the absence of hydroxy acids. This evidence further substantiates the likelihood of the hydroxy body, associated with the fatty acids from the seed fat, being sphingosin.

AG/A/60F

Fraction	*Wt.* (*g.*)	*Mono-ethenoid esters*		*Di-ethenoid esters*		*Tri-ethenoid esters*	"*Non-sap*"
		C_{16}	C_{18}	C_{16}	C_{18}	C_{18}	
XXIII	4·256	1·808	—	1·333	1·022	—	0·093
XXIV	9·609	1·447	0·086	—	7·988	—	0·088
XXV	7·422	0·756	0·435	—	6·155	—	0·076
XXVI	11·288	0·940	1·332	—	8·456	0·459	0·101
XXVII	8·539	—	4·459	—	1·197	2·775	0·108
XXVIII	2·511	—	1·085	—	0·565	0·558	0·303
XXIX	9·415	—	4·213	—	2·373	1·345	1·484
Total	53·040	4·951	11·610	1·333	27·756	5·137	2·253

Fraction XXIII was calculated to contain hexadecadienoic acid, being the only way of satisfactorily explaining the values of the chemical constants of the ester fraction. The constitution of this acid was proved by oxidation with potassium permanganate. (See No. III of this series of papers.)

These calculations gave the weight of the various esters in the total distilled samples, but allowance had to be made for slight losses during crystallization and esterification and also for samples removed for analysis.

The corrected weights of the ester fractions and their equivalent weights of fatty acids are given in the table below. (Table I).

Thus, the fatty acid composition of the seed fat, was corrected to the nearest 0·1 per cent as shown in Table II.

The calculated neutralization equivalent of this mixture of fatty acids is 278·1 which, is the same as the mean molecular weight of fatty acids calculated from the acid and saponification values and the percentage of unsaponifiable matter of the fat itself.

The neutralization equivalent as determined on the mixed fatty acids was found to be 284·3 which is considerably higher than the above calculated values. This was determined on fatty acids containing the hydroxy body which was shown to be "non-saponifiable" and this would have had the effect of increasing the neutralization equivalent.

Since there was 2·298 g. "non-saponifiable" matter associated with 100 g. of fatty

TABLE I

	Esters (corrected)			*Fatty acids*			
	AG /A /30	AG /A /60	AG /A /60F	AG /A /30	AG /A /60	AG /A /60F	*Total*
Myristic	0·073	—	—	0·065	—	—	0·065
Palmitic	10·280	9·084	—	9·262	8·612	—	17·874
Stearic	8·502	0·063	—	7·739	0·060	—	7·799
Arachidic	2·604	—	—	2·390	—	—	2·390
Behenic	1·419	—	—	1·311	—	—	1·311
Tetra-decenoic	0·006	—	—	0·006	—	—	0·006
Palmitoleic	4·247	0·841	5·882	3·824	0·799	5·556	10·179
Oleic	6·944	14·040	13·780	6·313	13·380	13·100	32·796
Eicosenoic	2·170	—	—	1·990	—	—	1·990
Hexadeca-dienoic	—	—	1·589	—	—	1·496	1·496
Linoleic	—	27·725	32·895	—	26·396	31·298	57·690
Linolenic	—	—	6·092	—	—	5·789	5·789
"Non-sap."	0·197	0·473	2·563	0·197	0·473	2·563	3·203
Total	36·442	52·226	62·810	33·100	49·720	59·802	142·622

acids, the neutralization equivalent of the fatty acids alone

$$= 284 \cdot 3 \times \frac{100}{102 \cdot 30}$$
$$= 278 \cdot 0$$

The calculated iodine value of the fatty acid mixture is 119·9 g. I_2/100 g. which is in good agreement with the value of 120·0 g. I_2/100 g. determined on the mixed fatty acids.

The composition of another sample of mixed fatty acids was later determined using the alkali isomerisation and spectrophotometric technique of Hilditch, Morton and Riley.[7]

$$E^{1\%}_{1\,\text{cm.}}\quad 268\ \text{m}\mu = 33 \cdot 00 \text{ (after alkali isomerization)}$$

$$E^{1\%}_{1\,\text{cm.}}\quad 234\ \text{m}\mu = 426 \cdot 5 \text{ (after alkali isomerization)}$$

Iodine value (Wijs) = 121·9

Percentage linolenic acid = 6·2
Percentage diethenoid acids (as linoleic) = 43·2
Percentage monoethenoid acid (as oleic) = 30·0
Percentage saturated acids (by difference) = 20·6

The agreement between these values and those obtained by the ester fractionation procedure is within the limits of accuracy claimed for determinations by this latter method.

TABLE II

	Wt. percentage		*Mol. percentage*	
Saturated		21·0		21·5
Myristic	trace		trace	
Palmitic	12·8		13·8	
Stearic	5·6		5·5	
Arachidic	1·7		1·5	
Behenic	0·9		0·7	
Mono-ethenoid		32·2		32·3
Tetradecenoic	trace		trace	
Palmitoleic	7·3		7·9	
Oleic	23·5		23·1	
Eicosenoic	1·4		1·3	
Di- and tri-ethenoid		46·8		46·2
Hexadecadienoic	1·1		1·2	
Linoleic	41·5		40·8	
Linolenic	4·2		4·2	
	100·0		100·0	

The unsaponifiable fraction of the seed fat

The unsaponifiable matter was isolated by the standard S.P.A. method[8] and the percentages of sterols, α-glyceryl ethers, and saturated and unsaturated hydrocarbons determined as described by Karnovsky, Rapson *et al.*[9] The carotenoids present in the unsaponifiable matter were determined spectrophotometrically[10] and found to be negligible.

The following is then the composition of the unsaponifiable fraction of the seed fat:—

	Weight per cent
Saturated hydrocarbons	16
Unsaturated hydrocarbons (as squalene)	2
Sterols (as sitosterol)	49
α-glyceryl ethers (as selachyl alcohol)	3
Carotenoids	nil
	70

The sterol digitonides, after separation, were split according to the method of Dam[11] and the free sterols extracted. These crystallized in tufts of needles from ether (M.P. 139–139·2° C.) and with the Liebermann-Borchard reagent produced a greenish violet colour. From this data, it was concluded that sitosterol was present almost to the exclusion of other sterols.

The hydroxyl value of the unsaponifiable matter was 140·0 mg. KOH/g. From this, was calculated the hydroxyl value of the 30 per cent unaccounted for in the above analysis, and found to be 153·0 mg. KOH/g.

This fraction thus most probably consists of choline from the phosphatides, and also the higher aliphatic alcohols.

In conclusion, the authors wish to record their thanks to Professor H. Stephen for his interest and encouragement during the course of this work and to the Council for Scientific and Industrial Research for a Grant to one of us (G.S.H.).

University of the Witwatersrand,
Johannesburg.

Received February 2, 1952.

REFERENCES

[1] Verdoorn, *J. Dept. Agr. Union S. Afr.*, 1924, **8**, 415.
[2] Bailey, "Industrial oil and fats products", Interscience Publishers, New York, 1945, p. 194.
[3] Hilditch, "Industrial fats and waxes", "Bailliere, Tindall and Cox, London, 3rd. Ed., 1949, p. 82
[4] Schoeman, M.Sc. Thesis, University of the Witwatersrand, 1946, p. 26.
[5] Hilditch, "The chemical constitution of natural fats", Chapman and Hall, London, 2nd Ed., 1949, pp. 474–479.
[6] Hilditch, op. cit. p. 509.
[7] Hilditch, Morton and Riley, *Analyst*, 1945, **70**, 68.
[8] B.S. 684, 1950, p. 28.
[9] Karnovsky, Rapson, *et al.*, *J. Soc. Chem. Ind.*, 1948, **67**, 106.
[10] Black, Harris and Schwartz, *J. S. Afr. Chem. Inst.*, New Series, 1949, **2**, 118.
[11] Dam, *Biochem. Z.*, 1928, **194**, 177.

STUDIES OF THE FATS FROM INDIGENOUS SOUTH AFRICAN PLANTS

II. THE SEED POD FAT OF ACACIA GIRAFFAE (KAMEELDOORN)

by

G. S. HARRISON and F. HAWKE

OPSOMMING

'n Monster van die saadhuls vet van *Acacia giraffae* is geëkstraheer en die fisiese en chemiese konstante bepaal asook die samestemmings van die vetsure en die onverseepbare materiaal.

Die saadhulse bevat omtrent 1·2 persent vet, wat die volgende konstante toon:—

$$\text{S.G. } d\,{}^{25^\circ}_{4^\circ} = 0{\cdot}9373;\ [n]\,{}^{70^\circ}_{D} = 1{\cdot}4599$$

Suurgetal = 57·3; versepingsgetal = 143·2; joodgetal = 69·0; hidroksielgetal = 30·2; onverseepbare materiaal = 26·0 persent; Riechert-Meisel getal = 0·4; Polenske getal = 0·3. Die vet lewer 70·8 persent vetsure wat die volgende samestelling toon (bereken as gewig persent): miristiensuur = 0·8; Palmitiensuur = 17·0; steariensuur = 9·1; arachiensuur = 8·3; behensuur = 0·1; tetradeceniensuur = spoor; palmitoliesuur = 10·4; oliesuur = 29·3; eicoseniensuur = 4·9; hexadecadiëniensuur = 1·1; linolsuur = 19·0.

SUMMARY

A sample of the seed pod fat of *Acacia giraffae* was extracted and the physical and chemical constants determined, together with the fatty acid composition and the composition of the unsaponifiable matter.

The seed pods contain about 1·2 per cent of fat having the following constants:—

$$\text{Sp. gr. } d\,{}^{25^\circ}_{4^\circ} = 0{\cdot}9373;\ [n]\,{}^{70^\circ}_{D} = 1{\cdot}4599.$$

Acid val. = 57·3; sap. val. = 143·2; I.V. = 69·0; hydroxyl val. = 30·2; unsap. = 26·0 per cent; R.M. val. = 0·4; Polenske val. = 0·3; the fat yields 70·8 per cent of fatty acids which have the following composition (weights per cent) myristic acid = 0·8; palmitic = 17·0; stearic 9·1; arachidic = 8·3; behenic = 0·1; tetradecenoic = trace; palmitoleic = 10·4; oleic = 29·3; eicosenoic = 4·9; hexadecadienoic = 1·1; linoleic = 19·0.

General characteristics of the fat

The fat from the seed pods of *Acacia giraffae* is a solid very dark green fat with a not unpleasant herb-like odour. After removal of the seeds, the pods were finely ground in a laboratory hammer mill and the fat extracted in the stainless steel extractor used for the seeds.[1]

Physical constants

$$\begin{aligned}
\text{Specific gravity } d\,{}^{25^\circ}_{4^\circ} &= 0{\cdot}9373 \text{ (solid)}\\
d\,{}^{40^\circ}_{4^\circ} &= 0{\cdot}9203 \text{ (solid)}\\
d\,{}^{70^\circ}_{4^\circ} &= 0{\cdot}8792 \text{ (liquid)}\\
\text{Refractive index } [n]\,{}^{65^\circ}_{D} &= 1{\cdot}4618\\
[n]\,{}^{70^\circ}_{D} &= 1{\cdot}4599\\
[n]\,{}^{75^\circ}_{D} &= 1{\cdot}4582
\end{aligned}$$

Temperature coefficient of refractive index	= −0·00036 per ° C.
Optical activity	negligible
Surface tension	= 35·10 dynes/cm. at 70° C.
Interfacial tension with water	= 8·91 dynes/cm. at 70° C.

The fat was found to have a very short melting range, changing from solid to completely molten over the range 63·0–63·8° C.; but it began softening at about 35° C. and was plastic between 55° C. and its melting point.

The temperature coefficient of the refractive index was observed to be slightly lower than that given for fats at temperatures near 70° C. This, however, is not of much significance.

As with the seed fat,[1] there was a considerable and abnormal lowering of the interfacial tension with water compared with the surface tension. The emulsions formed with water, however, were not as stable as in the case of the seed fat due to the appreciably higher value of 8·91 dynes/cm. This property was again shown to be due to mucilaginous material and phosphatides since their removal by treating the fat with boiling sodium chloride brine resulted in an increase of the interfacial tension to 24·2 dynes/cm. at 70° C.

Chemical constants

Acid value	= 57·3 mg. KOH/g.
Saponification value	= 143·2 mg. KOH/g.
Iodine value (Wijs)	= 69·0 g. I_2/100 g.
Hydroxyl value	= 30·2 mg. KOH/g.
Unsaponifiable matter	= 26·0 per cent.
Reichert Meisel value	= 0·4
Polenske value	= 0·3
Fatty acids	= 70·8 per cent.
Mean molecular weight of fatty acids	= 305·3 (by direct titration).

The mean molecular weight of the fatty acids calculated from the formula[2]:—

$$m \frac{1 \cdot 683 \times 10^3 (100 - u) - 38E}{3S}$$

was found to be 276·3. This is much lower than that determined by direct titration but this discrepancy was explained in the light of further investigations.

The fatty acid composition

This was determined on a 150-g. sample of the fat using a combination of low temperature crystallization, and ester fractionation.

After saponification of the fat and extraction of the unsaponifiable matter, the soaps were split and the fatty acids extracted with ether. This yielded 106·3 g. of fatty acids, of which 101 g. were dissolved in 1,000 ml. of dry redistilled acetone and boiled under reflux until all the fatty acids had dissolved. The solution was cooled to room temperature (25° C.) when it was allowed to stand for 18 hours before filtering. From this filtration, 100·0 g. of solvated crystals were obtained which yielded 16·6 g. of fatty acids (PAG/A/+25).

The filtrate, together with 100 ml. dry acetone used to wash the crystals, was then crystallized at 0° C. for 18 hours and filtered yielding 32 g. of solvated crystals. On removing the solvent, 11·7 g. of fatty acids (PAG/A/O) were obtained.

This filtrate was crystallized at — 35° C. for six hours with continuous stirring, giving 130 g. of solvated crystals, from which 33·4 g. of fatty acids (PAG/A/— 35) were obtained. From the filtrate, 38·9 g. of fatty acids (PAG/A/—35F) were recovered.

The fatty acid fractions were esterified with anhydrous methanol and fractionated in an "E.H.P." column.[3]

After esterification of both PAG/A/+25 and PAG/A/O, large quantities of a fluffy precipitate settled out on cooling; this was filtered off before dilution and extraction of the esters with ether. This was then set aside for further investigation.

The following tables give the results of the ester-fractionations:—

PAG/A/—35F

Weight of esters distilled = 36·31 g.
Saponification equivalent = 297·3
Saponification value = 188·5
Iodine value = 120·9

Fraction	*Weight (g.)*	*Sap. equiv.*	*Sap. val.*	*Iod. val.*
I	4·201	267·6	209·6	107·6
II	3·234	286·9	195·5	125·7
III	4·422	289·0	194·1	128·1
IV	6·727	291·9	192·2	129·8
V	3·418	292·2	192·0	127·5
VI	2·008	295·2	190·0	124·0
VII	2·114	293·0	191·4	123·7
VIII	2·371	291·0	192·8	121·7
IX	2·493	287·6	195·0	97·83
X	5·075	364·4	153·7*	86·96
Total	36·063			

* 0·1347 Gram solid separated out, during the determination of saponification values, per g. esters saponified. This was determined by filtering the neutralized saponification mixture through a sintered glass crucible, and washing alternately with N/2 hydrochloric acid and ether to ensure the removal of any fatty acids present as their potassium soaps. After a final washing with ether, the crucible and contents were dried in a vacuum desiccator and weighed.

The saponification and iodine values of the mixed esters were calculated from the saponification and unsaturation products. These were found to differ considerably from those determined on mixed esters, due to oxidation of the column and flask residues. The values for these two fractions (IX and X), therefore, were corrected as described in a previous communication[1] to allow for this oxidation.

The iodine values for fractions IX and X became 121·0 and 103·3 respectively and the corresponding saponification values of 190·0 and 150·8.

The values of 103·3 for the iodine value and 150·8 for the saponification value of fraction X were arrived at by extrapolating on the curves of the respective values versus weights of ester and then calculating the values for the fraction allowing for the separated solids. These were found to have zero iodine value and to be "non-saponifiable."

The following are the calculated values for the constants of the mixed esters before and after allowing for oxidation:

	Direct determination	*Calculated*	
		Before allowing for oxidation	*After allowing for oxidation*
Saponification value	188·5	189·2	188·6
Iodine value	120·9	117·5	120·8

PAG/A/-35

Weight of esters distilled = 30·61 g.
Saponification equivalent = 308·8
Saponification value = 192·6
Iodine value = 36·13

Fraction	*Weight (g.)*	*Sap. equiv.*	*Sap. val.*	*Iod. val.*
XI	5·085	267·9	209·4	9·92
XII	4·874	273·3	205·3	14·00
XIII	2·330	277·2	202·4	17·23
XIV	3·068	281·1	199·5	42·37
XV	3·312	290·5	193·1	72·49
XVI	3·658	295·5	189·9	77·07
XVII	3·009	308·7	181·8	37·47
XVIII	5·015	342·7	163·5*	27·64
Total	30·351			

* 0·0936 Gram solid separated out per g. of esters during the determination of saponification values.

The saponification and iodine values of the mixed esters were calculated as before. In this case, due to the absence of di-unsaturated acids, oxidation was not very severe and only a slight correction was necessary for the iodine values of the flask and column residues.

The iodine values of fractions XVII and XVIII were thus adjusted to 42·0 and 31·7 respectively, giving the following calculated values for the constants of the mixed esters.

	Direct determination	*Calculated*	
		Before allowing for oxidation	*After allowing for oxidation*
Saponification value	192·6	192·8	192·8
Iodine value	36·13	35·20	36·1

PAG/A/O

Weight of esters distilled = 8·64 g.
Saponification equivalent = 308·2
Saponification value = 172·9
Iodine value = 20·6

Fraction	*Weight (g.)*	*Sap. equiv.*	*Sap. val.*	*Iod. val.*
XIX	1·328	283·0	198·3	29·90
XX	1·919	304·3	184·4	41·02
XXI	1·157	310·8	180·5	22·17
XXII	1·705	313·9	178·6	3·49
XXIII	2·528	395·4	141·9*	10·48
Total	8·637			

* 0·1728 Gram "non-saponifiable" matter separated out per g. of esters during the saponification value determination.

The calculated saponification and iodine values of the mixed esters were computed and found to agree well with the determined values. This indicated that little or no oxidation of the residual fractions had taken place and corrections, therefore, were unnecessary.

The following is a comparison of the values:

	Direct determination	*Calculated*
Saponification value	172·9	172·7
Iodine value	20·6	20·5

PAG/A/+25

Weight of esters distilled = 8·68 g.
Saponification equivalent = 321·2
Saponification value = 174·5
Iodine value = 59·28

Fraction	*Weight (g.)*	*Sap. equiv.*	*Sap. val.*	*Iod. val.*
XXIV	1·336	275·6	203·5	55·81
XXV	2·145	286·3	196·0	82·69
XXVI	0·673	289·7	193·6	85·35
XXVII	1·339	315·7	197·0	46·39
XXVIII	3·154	395·6	141·8*	27·99
Total	8·647			

* 0·2210 Gram solids separated out per g. esters during saponification value determination.

From the calculated values for the saponification and iodine values of the mixed esters, it was concluded that oxidation of the two residual fractions had taken place. Thus the iodine value of fractions XXVII and XXVIII were corrected to 70·0 and 34·7 respectively and the corresponding saponification values to 192·0 and 136·1. These changed the calculated value for the mixed esters as follows:

	Direct determination	*Calculated*	
		Before allowing for oxidation	*After allowing for oxidation*
Saponification value	174·5	177·2	174·5
Iodine value	59·28	53·26	59·3

Composition of the ester fractions

PAG/A/—35F

Fraction	*Weight (g.)*	*Saturated esters*		*Mono-ethenoid esters*			*Di-ethenoid esters*		*"Non-sap."*
		C_{14}	C_{16}	C_{16}	C_{18}	C_{20}	C_{16}	C_{18}	
I	4·201	0·256	—	3·307	—	—	0·638	—	—
II	3·234	—	0·068	0·679	0·980	—	0·132	1·375	—
III	4·422	—	0·049	0·694	1·481	—	0·133	2·065	—
IV	6·727	—	0·800	—	1·707	—	—	4·220	—
V	3·418	—	0·376	—	1·019	—	—	2·023	—
VI	2·008	—	0·022	—	1·074	—	—	0·912	—
VII	2·114	—	0·197	—	0·838	—	—	1·079	—
VIII	2·371	—	0·370	—	0·650	—	—	1·351	—
IX	2·493	—	—	—	1·477	—	—	1·016	—
X	5·075	—	—	—	—	2·475	—	1·916	0·684
Total	36·063	0·256	1·882	4·680	9·226	2·475	0·903	15·957	0·684

PAG/A/—35

Fraction	*Weight (g.)*	*Saturated esters*				*Mono-ethenoid esters*			*"Non-sap."*
		C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	
XI	5·085	0·407	4·618	—	—	0·009	0·051	—	—
XII	4·874	—	3·658	0·453	—	—	0·595	0·141	—
XIII	2·330	—	1·404	0·487	—	—	0·291	0·148	—
XIV	3·068	—	0·963	0·663	—	—	0·748	0·694	—
XV	3·312	—	0·149	0·414	—	—	0·539	2·210	—
XVI	3·658	—	0·165	0·201	—	—	—	3·292	—
XVII	3·009	—	—	1·138	0·394	—	—	1·477	—
XVIII	5·015	—	—	1·436	1·255	—	—	1·854	0·470
Total	30·351	0·407	10·984	4·792	1·649	0·009	2·224	9·816	0·470

PAG/A/O

Fraction	*Weight (g.)*	*Saturated esters*				*Mono-ethenoid esters*			*"Non-sap."*
		C_{16}	C_{18}	C_{20}	C_{22}	C_{16}	C_{18}	C_{20}	
XIX	1·328	0·470	0·416	—	—	0·203	0·239	—	—
XX	1·919	—	0·511	0·489	—	—	0·919	—	—
XXI	1·157	—	0·298	0·561	—	—	0·298	—	—
XXII	1·705	—	0·721	0·977	—	—	0·007	—	—
XXIII	2·528	—	—	1·669	0·080	—	—	0·342	0·437
Total	8·637	0·470	1·946	3·696	0·080	0·203	1·463	0·342	0·437

PAG/A/+25

Fraction	*Weight (g.)*	*Saturated esters*			*Mono-ethenoid esters*			*"Non-sap."*
		C_{16}	C_{18}	C_{20}	C_{16}	C_{18}	C_{20}	
XXIV ...	1·336	0·422	0·106	—	0·584	0·224	—	—
XXV ...	2·145	0·060	0·086	—	0·687	1·312	—	—
XXVI ...	0·673	0·005	0·012	—	0·145	0·511	—	—
XXVII ...	1·339	0·194	0·051	—	—	1·094	—	—
XXVIII ...	3·154	—	0·233	0·947	—	0·167	1·111	0·696
Total	8·647	0·681	0·488	0·947	1·416	3·308	1·111	0·696

These calculations gave the weights of the various esters in the total distilled samples, but allowances had to be made for slight losses during crystallization, and esterification and also for samples removed for analysis.

The corrected weights of the ester fractions and their equivalent weights of fatty acids are given in Table I.

TABLE I

	Methyl esters				*Total*	*Total fatty acids*
	PAG/A/+25	*PAG/A/O*	*PAG/A/−35*	*PAG/A/−35F*		
Myristic	—	—	0·473	0·291	0·764	0·72
Palmitic	0·884	0·610	12·750	2·141	16·385	15·53
Stearic	0·633	2·525	5·563	—	8·721	8·31
Arachidic	1·228	4·797	1·914	—	7·939	7·60
Behenic	—	0·104	—	—	0·104	0·10
Tetra-decenoic	—	—	0·010	—	0·010	0·01
Palmitoleic	1·837	0·264	2·582	5·322	10·005	9·48
Oleic	4·291	1·900	11·400	10·490	28·081	26·75
Eicosenoic	1·442	0·444	—	2·815	4·701	4·50
Hexadeca-dienoic	—	—	—	1·036	1·036	0·98
Linoleic	—	—	—	18·160	18·160	17·30
"Non-sap."	0·903	0·567	0·546	0·778	2·794	2·79
Total	11·218	11·211	35·238	41·033	98·700	94·07

The fatty acid composition of the seed pod fat corrected to the nearest 0·1 per cent is shown in Table II.

TABLE II

	Weight per cent		*Molar per cent*	
Saturated acids		35·3		35·5
Myristic	0·8		1·0	
Palmitic...	17·0		18·3	
Stearic	9·1		8·8	
Arachidic	8·3		7·3	
Behenic	0·1		0·1	
Monoethenoid acids		44·6		44·6
Tetradecenoic	Trace		Trace	
Palmitoleic	10·4		11·3	
Oleic	29·3		28·8	
Eicosenoic	4·9		4·5	
Di-ethenoid acids		20·1		19·9
Hexadecadienoic	1·1		1·2	
Linoleic...	19·0		18·7	
	100·0		100·0	

The neutralization equivalent of this mixture of fatty acids was calculated to be 276·4. This agrees well with the value of 276·3 for the mean molecular weight of the fatty acids calculated from the acid and saponification values and the percentage unsaponifiable matter of the fat itself. However, there is a considerable difference between these values and that determined by direct titration of the fatty acids which was 305·3.

This was determined on "fatty acids" containing large quantities of "non-saponifiable" matter which was later separated during the esterification process and the determination of saponification values on ester fractions. When corrected for this "non-saponifiable" matter, the value is very close to calculated values.

Associated with 91·28 g. of fatty acids, there was a total of 2·79 g. "non-saponifiable" matter removed during saponification and also 6·93 g. removed during esterification.

Hence, the neutralization equivalent of the fatty acids

$$= 305 \cdot 3 \times \frac{91 \cdot 28}{101 \cdot 00}$$

$$= 276 \cdot 0$$

The calculated iodine value for the mixed fatty acids is 79·4 g. I_2/100 g. while that determined on the fatty acids was 71·9 g. I_2/100 g. However, when corrected for the "non-saponifiable" matter, of zero iodine value, it becomes 79·7 g. I_2/100 g. which is in good agreement with the calculated value.

The composition of another sample of fatty acids was later determined using the alkali isomerization and spectrophotometric technique of Hilditch, Morton, and Riley.[4]

$$E^{1\,\%}_{1\,\mathrm{cm}} \quad 268\ \mathrm{m}\mu = 2 \cdot 73 \quad \text{(after alkali isomerization)}$$

$$E^{1\,\%}_{1\,\mathrm{cm}} \quad 234\ \mathrm{m}\mu = 205 \cdot 8 \quad \text{(after alkali isomerization)}$$

I.V. $= 79 \cdot 2$ g. I_2/100 g.

Percentage linolenic	=	0·5
Percentage diethenoid acids (as linoleic)	=	22·3
Percentage monoethenoid acids (as oleic)	=	41·6
Percentage saturated acids (by difference)	=	35·6
		100·0

This fatty acid composition agrees well with that determined by ester fractionation

The unsaponifiable fraction of the seed pod fat

The unsaponifiable matter was isolated by the standard S.P.A. method,[5] and the percentage of sterols, α-glyceryl ethers and saturated and unsaturated hydrocarbons determined as described by Karnovsky, Rapson, *et al.*[6]

The carotenoids present in the unsaponifiable matter were determined spectrophotometrically[7] and found to be negligible.

The following then, is the composition of the unsaponifiable fraction of the seed fat:—

	Weight per cent
Saturated hydrocarbons	9·1
Unsaturated hydrocarbons (as squalene)	0·3
Sterols (as sitosterol)	15·8
α-glyceryl ethers (as selachyl alcohol)	1·7
	26·9

The sterol digitonides, after separation, were split according to the method of Dam[8] and the free sterols extracted. These crystallized in tufts of short needles from ether (M.P. 139–139·6° C.) and with the Liebermann-Borchard reaction produced a greenish violet colour. From this data, it was concluded that sitosterol was present almost to the exclusion of other sterols.

From the hydroxyl value of the unsaponifiable matter (93·0) the hydroxyl value of the 73·1 per cent unaccounted for in the above analysis was calculated to be 82·9.

This hydroxyl value, according to Karnovsky, *et al.*[6] is indicative of the presence of fatty alcohols in the unsaponifiable matter; the low value indicates the presence of other non-hydroxylated substances.

The "non-saponifiable" matter

The solids separating out from the fatty acid fractions on esterification, and during the determination of saponification values on the flask residues from the ester fractionation, were boiled with dilute sulphuric acid. After this treatment all the above gave positive reactions for carbohydrates with Molisch's test, indicating the possibility of their being polysaccharides.

The presence of polysaccharides or other carbohydrates in the "fatty acids" probably explains the high degree of unsaturation in the least soluble fatty acids in fraction PAG/A/+25. This would have been expected to contain only the most saturated of the fatty acids.

Rewald[9] indicates that carbohydrates are associated with fatty acids and phosphatides in a loose combination but without the formation of any stable compound. The acids most commonly associated with carbohydrates are the unsaturated C_{16}–C_{20} acids which are present to a considerable extent in PAG/A/+25. Thus it would appear that the carbohydrates remained associated with the fatty acids and decreased their solubility in acetone giving unexpected quantities of unsaturated acids separating out at room temperature.

In conclusion, the authors wish to record their thanks to Professor H. Stephen for his interest and encouragement during the course of this work and to the Council for Scientific and Industrial Research for a grant to one of us (G. S. H.)

University of the Witwatersrand,
Johannesburg.

Received February 2, 1952.

[1] Harrison and Hawke, No. 1 of this series: "The seed fat of *Acacia giraffae*."
[2] Schoeman, M.Sc. Thesis, University of the Witwatersrand, 1946, p. 26.
[3] Hilditch, "The chemical constitution of natural fats", Chapman and Hall, London, 2nd Ed., 1949, pp. 474-479.
[4] Hilditch, Morton and Riley, *Analyst*, 1945, **70**, 68.
[5] B.S. 684, 1950, p. 26.
[6] Karnovsky, Rapson *et al.*, *J. Soc. Chem. Ind.*, 1948, **67**, 106.
[7] Black, Harris and Schwartz, *J. S. Afr. Chem. Inst.*, New series, 1949, **2**, 118.
[8] Dam, *Bio.-chem. Z.*, 1928, **194**, 177.
[9] Rewald, *Bio.-chem. Z.*, 1929, **211**, 199.

STUDIES OF THE FATS FROM INDIGENOUS SOUTH AFRICAN PLANTS

III. $\Delta^{9,12}$ HEXADECADIENOIC ACID: ITS CONSTITITION AND OCCURRENCE IN THE SEED FAT AND SEED POD FAT OF *ACACIA GIRAFFAE* (KAMEELDOORN)

by

G. S. HARRISON and F. HAWKE

OPSOMMING

Die teenwoordigheid is bewys van $\Delta^{9,12}$ hexadecadiëniensuur in die mengsel van vetsure van die saadvet en saadhulsvet van *Acacia giraffae*. Die samestelling van die suur was bewys deur oksiderende opbreking gevolg deur skeidingskromatografie van die dikarboksiel sure wat gevorm was.

SUMMARY

The presence of $\Delta^{9,12}$ hexadecadienoic acid in the mixed fatty acids of the seed fat and seed pod fat of *Acacia giraffae* has been shown. Its constitution was proved by destructive oxidation followed by partition chromatography of the dicarboxylic acids formed.

Occurrence in the seed fat

The presence of a hexadecadienoic acid was first suspected in ester fraction (methyl esters) number XXIII of the seed fat.[1] Its chemical constants were as follows:—

Saponification equivalent = 279·8 mg. KOH/g.
Iodine value (Wijs) = 143·9 g. I_2/100 g.
Thiocyanogen value = 93·03 g. I_2/100 g.

From the saponification and iodine values, it was decided that there would be a large percentage of unsaturated C_{16} acids in the fraction.

The iodine value indicated the presence of di- and, possibly, tri-ethenoid esters, but the presence of the latter was excluded by considering the compositions of the next two ester fractions. When these were calculated to include linolenic acid, in both cases, the amount was less than 0·1 per cent of the fraction. Hence it was unlikely that linolenic acid would be present in fraction XXIII.

The thiocyanogen value was higher than that of pure methyl linoleate (92·10) and to account for this, there would have had to be present in the fraction the esters of either linolenic or some di-ethenoid C_{16} acid. Since the presence of the former had already been shown to be unlikely, the fraction was assumed to contain the methyl ester of a hexadecadienoic acid, being the only way of explaining the values of the chemical constants satisfactorily.

Occurrence in the seed pod fat

In the case of fraction number I of the seed pod fat,[2] the evidence for the presence of a hexadecadienoic acid was even stronger. The chemical constants for this fraction of methyl esters were:—

Saponification equivalent = 267·6
Iodine value = 107·6

The iodine value was well above that of even methyl tetradecenoate and could only have been due to the methyl ester of a di-ethenoid acid.

The saponification equivalent was 0·8 unit below that of methyl palmitoleate so that the presence of methyl linoleate in the fraction was extremely unlikely. Hence, to account for both the low saponification equivalent and also the high iodine value, the presence of a methyl hexadecadienoate was postulated.

The constitution of the hexadecadienoic acid

Owing to the small percentage of the acid in both fats, it was not considered feasible to attempt to isolate it to determine its constitution by oxidation with potassium permanganate in acetone. Therefore, the two ester fractions mentioned above (AG/A/60F No. XXIII and PAG/A/—35F No. I) were selected for this purpose, having the largest proportions of the acid.

After reducing the excess permanganate with potassium bisulphite, the oxidation products were steam distilled and treated as shown in Figs. 1 and 2.

In the acid products from the steam distillate of both fractions, the presence of butyric acid was detected by its characteristic odour and further confirmed by means of its neutralization equivalent as shown.

From the oxidation of fraction No. XXIII, butyric acid could be obtained from any of the following esters:—

(i) $CH_3CH_2CH_2CH = CH\,CH_2CH = CH(CH_2)_7COOCH_3$
(ii) $CH_3CH_2CH_2CH = CH(CH_2)_{10}COOCH_3$
(iii) $CH_3CH_2CH_2CH = CH(CH_2)_3CH = CH(CH_2)_7COOCH_3$
(iv) $CH_3CH_2CH_2CH = CHCH_2CH = CH(CH_2)_9COOCH_3$
(v) $CH_3CH_2CH_2CH = CH(CH_2)_{12}COOCH_3$

Butyric acid could also be derived from fraction No. I by the oxidation of one of the following esters:—

(i) $CH_3CH_2CH_2CH = CH\,CH_2CH{=}CH(CH_2)_7COOCH_3$
(ii) $CH_3CH_2CH_2CH = CH(CH_2)_{10}COOCH_3$
(iii) $CH_3CH_2CH_2CH = CHCH_2CH = CH(CH_2)_5COOCH_3$
(iv) $CH_3CH_2CH_2CH = CH(CH_2)_8COOCH_3$

Which of the above esters was present in the fractions was indicated by the dicarboxylic acids present in the residues remaining in the steam distillation flasks.

The dicarboxylic acids were determined qualitatively by partition chromatography using a modification of the method of Bergemann, Keppler and Boekenoogen.[3]

The method was first tested with a synthetic mixture of one part each of azelaic and adipic acids and five parts each of succinic and malonic acids to determine the applicability of the method to the lower dicarboxylic acids. It was observed that the resolution of azelaic and adipic acids was extremely sharp (Fig. 3) but that succinic acid appeared only after 100 ml. of eluate had been collected and then only as a flat "hump" in the curve.

It was obvious that malonic acid would have taken even longer to appear and would have had an even flatter "hump" than succinic acid, Consequently, the method was modified to include elution with acetone after 170 ml. eluate had been collected. This figure was chosen because, in the above standardization, the succinic acid was observed to have been eluted completely by this quantity of eluent. From the curve (Fig. 3) the effect of this modification may be seen to be satisfactory and this method was adopted throughout the succeeding determinations.

Fig. 4 shows the four curves obtained from the various samples of dicarboxylic acids. In all cases the curves drawn with full lines are those of the acids obtained from the oxidation process. The broken lines are the curves obtained when small quantities of azelaic and malonic acids were added to the unknown acids to identify the peaks.

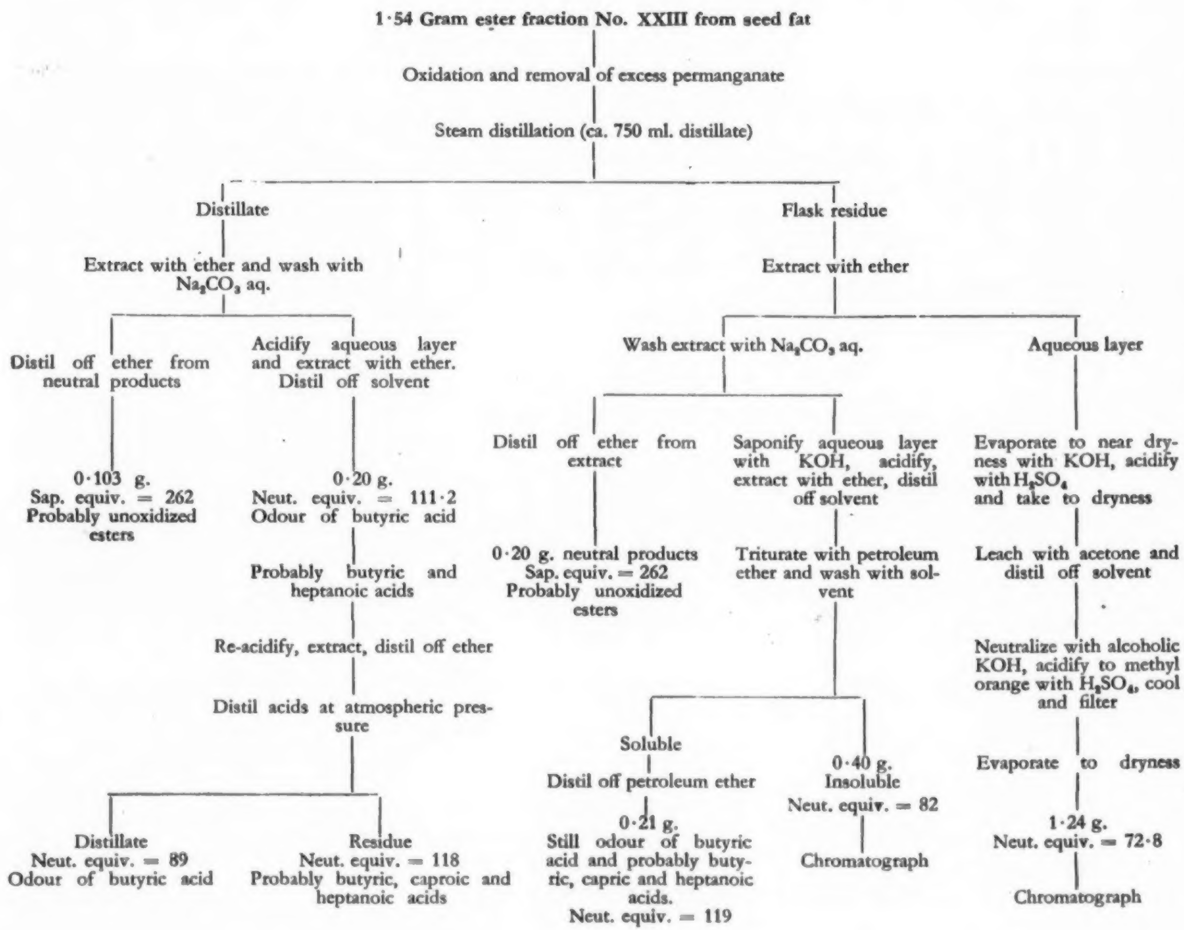

1·54 Gram ester fraction No. XXIII from seed fat
Oxidation and removal of excess permanganate
Steam distillation (ca. 750 ml. distillate)
Distillate
Extract with ether and wash with Na_2CO_3 aq.
Distil off ether from neutral products
0·103 g. Sap. equiv. = 262 Probably unoxidized esters
Acidify aqueous layer and extract with ether. Distil off solvent
0·20 g. Neut. equiv. = 111·2 Odour of butyric acid
Probably butyric and heptanoic acids
Re-acidify, extract, distil off ether
Distil acids at atmospheric pressure
Distillate Neut. equiv. = 89 Odour of butyric acid
Residue Neut. equiv. = 118 Probably butyric, caproic and heptanoic acids
Flask residue
Extract with ether
Wash extract with Na_2CO_3 aq.
Distil off ether from extract
0·20 g. neutral products Sap. equiv. = 262 Probably unoxidized esters
Saponify aqueous layer with KOH, acidify, extract with ether, distil off solvent
Triturate with petroleum ether and wash with solvent
Soluble
Distil off petroleum ether
0·21 g. Still odour of butyric acid and probably butyric, capric and heptanoic acids. Neut. equiv. = 119
0·40 g. Insoluble Neut. equiv. = 82
Chromatograph
Aqueous layer
Evaporate to near dryness with KOH, acidify with H_2SO_4 and take to dryness
Leach with acetone and distil off solvent
Neutralize with alcoholic KOH, acidify to methyl orange with H_2SO_4, cool and filter
Evaporate to dryness
1·24 g. Neut. equiv. = 72·8
Chromatograph

Figure 1.

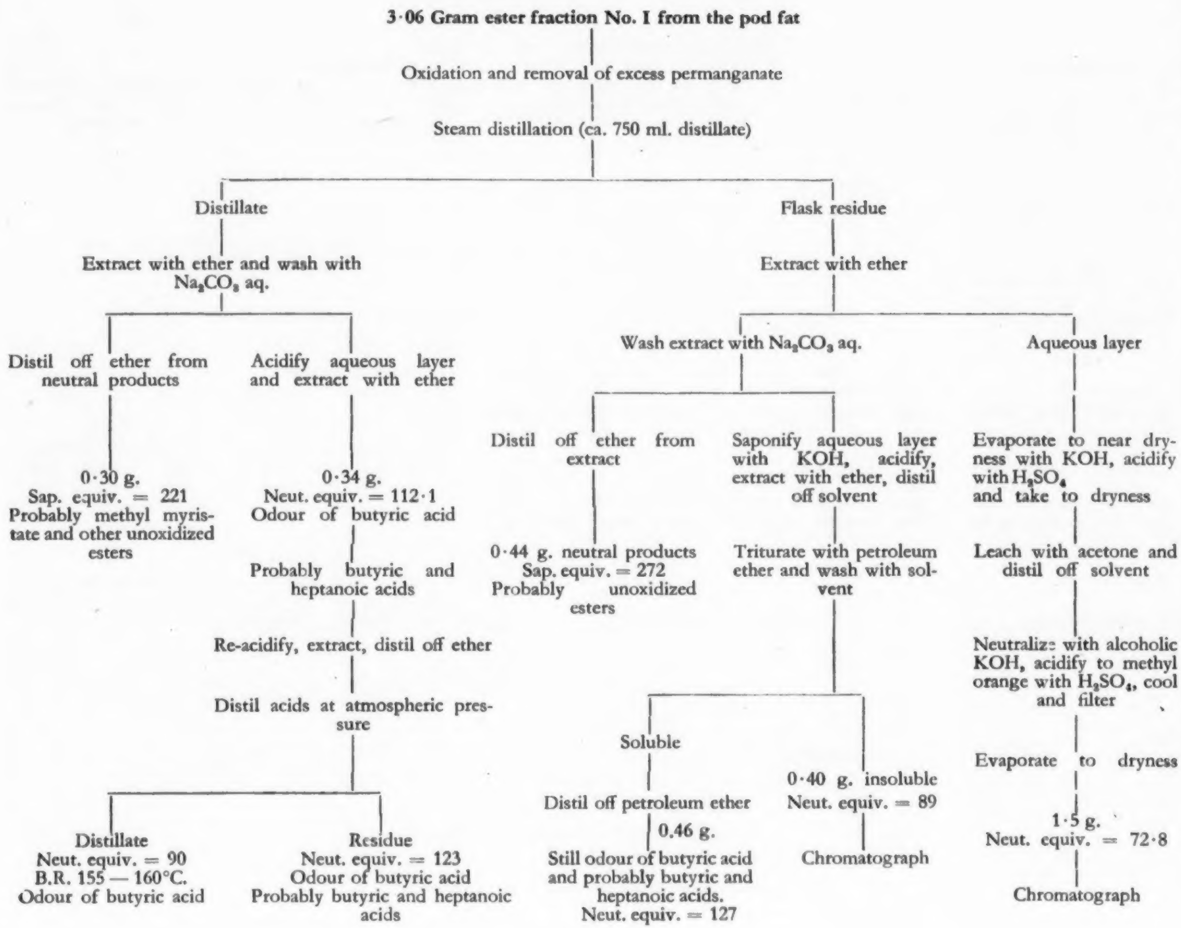

3·06 Gram ester fraction No. I from the pod fat
Oxidation and removal of excess permanganate
Steam distillation (ca. 750 ml. distillate)
Distillate
Flask residue
Extract with ether and wash with Na_2CO_3 aq.
Extract with ether
Distil off ether from neutral products
Acidify aqueous layer and extract with ether
Wash extract with Na_2CO_3 aq.
Aqueous layer
0·30 g.
Sap. equiv. = 221
Probably methyl myristate and other unoxidized esters
0·34 g.
Neut. equiv. = 112·1
Odour of butyric acid
Probably butyric and heptanoic acids
Re-acidify, extract, distil off ether
Distil acids at atmospheric pressure
Distillate
Neut. equiv. = 90
B.R. 155 — 160°C.
Odour of butyric acid
Residue
Neut. equiv. = 123
Odour of butyric acid
Probably butyric and heptanoic acids
Distil off ether from extract
0·44 g. neutral products
Sap. equiv. = 272
Probably unoxidized esters
Saponify aqueous layer with KOH, acidify, extract with ether, distil off solvent
Triturate with petroleum ether and wash with solvent
Soluble
Distil off petroleum ether
0,46 g.
Still odour of butyric acid and probably butyric and heptanoic acids.
Neut. equiv. = 127
0·40 g. insoluble
Neut. equiv. = 89
Chromatograph
Evaporate to near dryness with KOH, acidify with H_2SO_4 and take to dryness
Leach with acetone and distil off solvent
Neutralize with alcoholic KOH, acidify to methyl orange with H_2SO_4, cool and filter
Evaporate to dryness
1·5 g.
Neut. equiv. = 72·8
Chromatograph

Figure 2.

Figs. 4A and B are those obtained from the chromatography of the dicarboxylic acids extracted by ether from the residues in the steam distillation flasks.

Figs. 4C and D are the curves from the determinations on the dicarboxylic acids in the aqueous layers remaining after ether extraction of the residues in the distillation flasks.

The acids used to obtain Figs. 4A and C were from the oxidation of the pod fat fraction (No. I) and Figs. 4B and D from the seed fat fraction (No. XXIII).

In all the curves on dibasic acids from oxidation of the ester fractions, the first three to five ml. of eluate required appreciable quantities of alkali for neutralization. This was shown to be due to sulphuric acid which had not been washed from the acids since too much washing of the ether solutions resulted in the removal of the dicarboxylic acids as well.

These curves indicate the presence of azelaic and malonic acids to the exclusion of all other dicarboxylic acids in the oxidation products of the ester fractions.

The only possible ester from which only these two acids could be derived together with butyric acid, is $\Delta^{9,12}$ *hexadecadienoic acid.*

From this evidence, $\Delta^{9,12}$ hexadecadienoic acid was almost certainly present in the seed fat and seed pod fat of *Acacia giraffe*.

EXPERIMENTAL

The method of oxidation of the ester fractions was that used by Armstrong and Hilditch.[4]

The oxidation mixture, after the decomposition of excess potassium permanganate with sodium bisulphite and dilute sulphuric acid, was steam distilled. The steam and distillate and the residue in the flask were extracted with ether and the aqueous layer of the steam distillate was discarded, but that of the flask residue was reserved. It was thought that this latter layer would contain malonic acid as it is about 10 times as soluble in water as in ether while its partition coefficient between ether and water is 12:1 in favour of water.[5]

The dicarboxylic acids were then washed several times with petroleum ether to remove the monocarboxylic acids.[3]

Partition chromatographic analysis of the dicarboxylic acids

Silica gel was prepared according to Gordon *et al.*[6] 15 Gram of this silica gel was mixed with 20 ml. of aqueous phase obtained when three parts of ethanol, three parts of water and ten parts of benzene were thoroughly shaken and allowed to separate. The still powdery mixture was transferred to a glass tube of diameter 10 mm. and pressed together to form a column 20 cm. in length.

Twenty milligrams of the dicarboxylic acids were dissolved in 5 ml. of the benzene phase and poured on to the column. This was washed several times with 1 ml. portions of ethanol and then continuously with benzene phase. The percolate was collected in 1 ml. portions and each titrated separately with 0·05 N. ethyl alcoholic potassium hydroxide using phenol phthalein as indicator. After 170 ml. of percolate had been collected, the benzene phase was replaced with acetone as eluent. This eluted malonic acid from the column.

After chromatographing the dicarboxylic acids from the oxidation of the ester fractions, the curves of titration figure versus ml. of eluate were plotted and compared with the standardization curve to identify the peaks. The determinations were then repeated adding small quantities of known dicarboxylic acids (azelaic and malonic) to confirm the identities of the peaks (see Fig. 4).

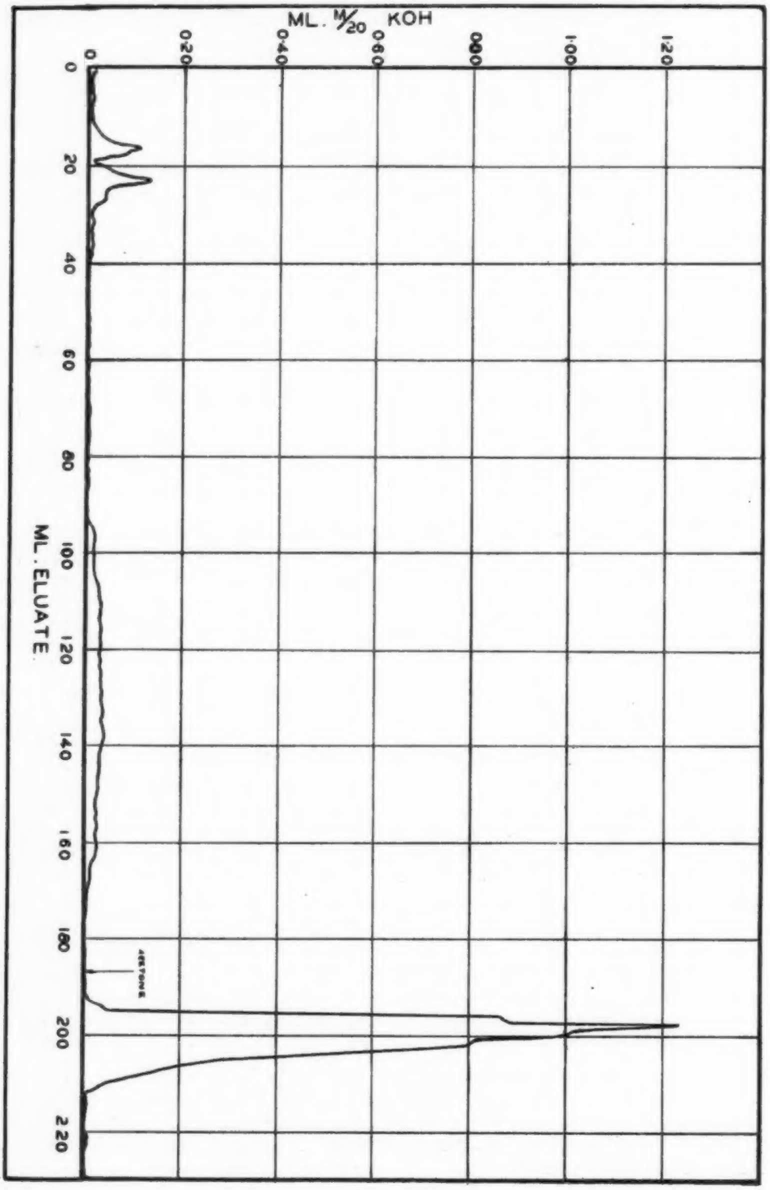

ML. M/20 KOH
0
0·20
0·40
0·60
0·80
1·00
1·20
0
20
40
60
80
100
120
140
160
180
200
220
ML. ELUATE.

Fig. 3

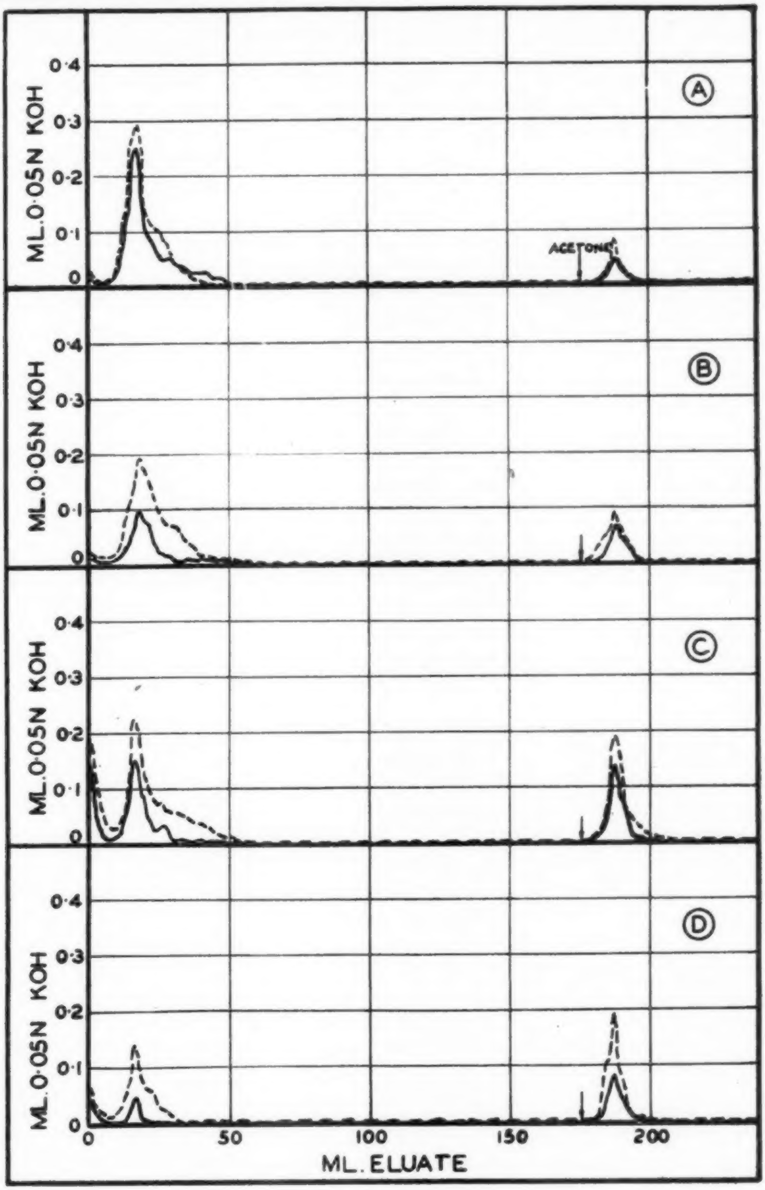


Fig. 4

The authors wish to record their thanks to Professor H. Stephen for his interest and encouragement during the course of this work and to Dr. W. S. Rapson for providing them with a sample of azelaic acid. One of the authors (G. S. H.) is indebted to the Council for Scientific and Industrial Research for a research grant.

University of the Witwatersrand,
Johannesburg.

Received February 2, 1952.

REFERENCES

[1] Harrison and Hawke, No. I of this series: "The seed fat of *Acacia giraffae*.
[2] Harrison and Hawke, No. II of this series: "The seed pod fat of *Acacia giraffae*."
[3] Bergemann, Keppler and Boekenoogen, Rec. Trav. Chim., 1950, **69**, 439.
[4] Armstrong and Hilditch, *J. Soc. Chem. Ind.*, 1925, **44**, 44T.
[5] International Critical Tables, Vol. 4, pp. 250 and 396.
[6] Gordon *et al.*, *Biochem. J.*, 1943, **37**, 80.

A NEW MANOMETRIC METHOD FOR THE DETERMINATION OF SULPHIDE ION IN SOLUTION

by

R. E. PRESS and K. A. MURRAY

OPSOMMING

Die konsentrasie van sulfied-ioon (S^{--}) kan vasgestel word deur sy katalitiese effek op die reaksie tussen asied (N_3^-) en jodium (I_2), terwyl die omvang van die reaksie bepaal word deur manometriese maat van die stikstof wat vrygestel word. Konsentrasie-reeks 0·5 tot 0·01 d.p.m., standaardafwyking ongeveer 10 persent van die sulfiedkonsentrasie.

SUMMARY

Sulphide (S^{-}) ion concentration may be determined by its catalytic effect on the reaction between azide (N_3^-) and iodine (I_2), the extent of reaction being determined by a manometric measure of the nitrogen evolved. Range of sample concentration 0·5 p.p.m. to 0·01 p.p.m., standard deviation approximately 10 per cent of sulphide concentration.

INTRODUCTION

Few methods for the determination of sulphide ion in low concentrations (1 to 0·01 p.p.m.) appear to be satisfactory. One valuable and comparatively simple method method is the methylene blue method.[1, 2] The authors found this method to be less sensitive than reported by R. Pomeroy.[1]

However, this may be due to the fact that observations were made visually with a simple colorimeter; it is possible that the method may be more sensitive if measurements are made with a photo-electric colorimeter or spectrophotometer.[2]

In view of the results reported by Goto Hidehiro and Shishiokawa Takanolu,[3] who used a fluorescence technique with Rhodamine B as indicator to follow the influence of sulphide ions on the reaction between iodine and azide, it was decided to investigate this latter reaction more fully.

The reaction between azide and iodine is catalysed by organic sulphides, hydro sulphides, thiosulphate, tetrathionates, sulphide and thiocyanate. Not however, by sulphite, sulphate and non-sulphur containing substances.[4, 5, 6, 7, 8]

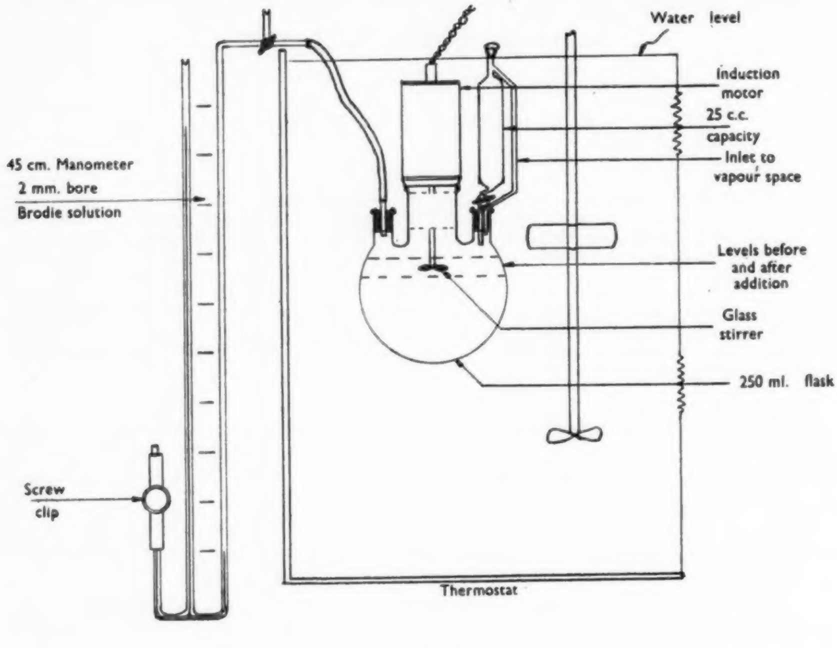
The reaction has been used to determine tetrathionates and cystine using a micro-manometric technique.[9, 10]

As a manometric technique is simple and inexpensive it was adopted in preference to that of flourescence and by its use a greater sensitivity was attained.

EXPERIMENTAL

Apparatus

The apparatus as shown in Fig. 1 functions on lines similar to a Warburg type, motion of the contents of the flask, however, is effected directly by means of a three-bladed glass stirrer driven by an induction motor sealed into the apparatus in such a manner that the contents of the flask is stirred only after addition of the side arm reactants; the liquid level rise due to this addition immersing the blades. In this manner the motor can attain temperature equilibrium and loss of hydrogen sulphide from the sample during preliminary "thermostating" is greatly reduced. The manometric change due to increase of "motor load" when the stirrer is immersed is small, as is the volume change due to mixing of the reactants. These and similar effects are accounted for by blank determinations.



Apparatus FIG. 1

The complete apparatus (motor, flask, and side-arm) with the exception of the manometers, is immersed in a large thermostat maintained at 25° C. × 0·02° C. The whole is used in conjunction with a thermobarometer.

For a sample volume of 250 cc. the constant of the apparatus was 0·107 cc./cm. at N.T.P. as determined by the Munzer and Neumann method.[11] The sensitivity is thus greatly enhanced, i.e., 0·000428 c.c./cm./ml. of sample, as compared with 0·003 c.c/cm./ml. for a typical Warburg apparatus. The large sample helps to avoid sampling difficulties.

Investigation of stirring rates

When studying a chemical reaction by a gas evolution method, the solution must be vigorously stirred so that the measured rate of gas evolution is independent of the stirring rate, within the sensitivity of the measuring system. Erroneous conclusions are drawn if this is not the case, as was demonstrated by Lamplough.[12]

If we form a known volume of gas by a rapid chemical reaction in a solution so as to supersaturate it, the gas will be evolved from solution at a rate which is a function of the supersaturation and stirring rate, for a given apparatus. This evolution rate is what is measured in all gas evolution methods.

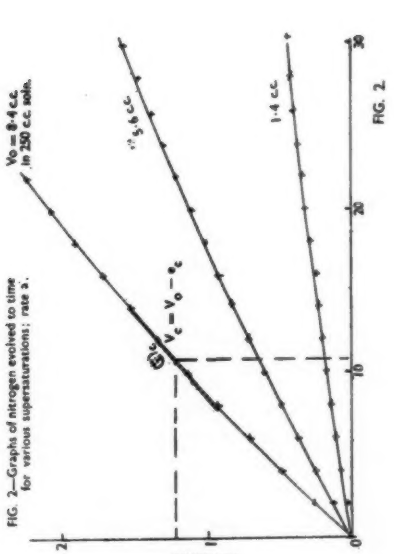


FIG. 2—Graphs of nitrogen evolved to time for various supersaturations; rate a.

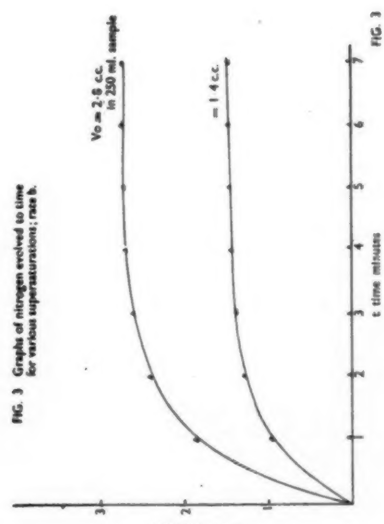


FIG. 3 Graphs of nitrogen evolved to time for various supersaturations; rate b.

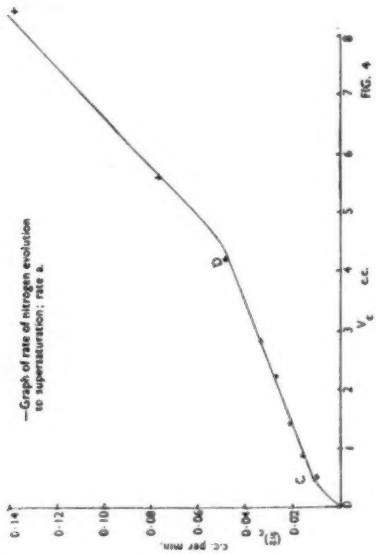


FIG. 4—Graph of rate of nitrogen evolution to supersaturation; rate a.

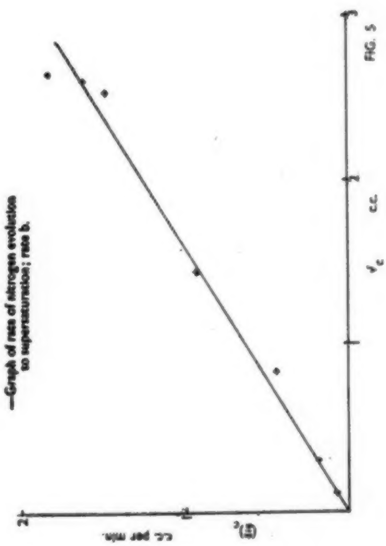


FIG. 5—Graph of rate of nitrogen evolution to supersaturation; rate b.

As the experiments here reported are concerned with the formation and evolution of nitrogen from aqueous solution, investigation of different stirring rates on a reaction giving rise to the rapid formation of nitrogen in solution, was undertaken.

A reaction convenient for this purpose was considered to be the reaction between sodium azide and ceric ammonium sulphate, viz:-

$$2\,NaN_3 + 2(NH_4)_2\,Ce(NO_3)_6 = 2\,Ce(NO_3)_3 + 2NaNO_3 + 4NH_4NO_3 + 3N_2$$

Investigation of the rate of evolution in this reaction (from salution) was carried out under conditions identical with those pertaining in the later kinetic studies here recorded.

Two series of experiments were performed at stirring rates of (*a*) 500 r.p.m. and (*b*) 2,800 r.p.m. (measured by means of a strobotac). Calculable volumes of nitrogen V were formed in solution and plots of gas evolved e to time t were made, Figs. 2 and 3; the evolution rate $\left(\frac{de}{dt}\right)_c$ at time t was measured and the volume of gas V_c (where $V_c = V_0 - e_c$) left in solution calculated. Plots of $\left(\frac{de}{dt}\right)_c$ to V_c are shown in Figs. 4 and 5.

In following a chemical reaction of relatively slow rate we can plot e against t and thus obtain at any time t_c, $\left(\frac{de}{dt}\right)_c$ and thus the volume stored in solution from Figs. 3 and 4 The volume of nitrogen formed by the reaction at time t is then given by the volume e_c plus V_c. We can then for a particular reaction plot both e against t and volume formed by the reactants $\mathcal{V}$ against t, e.g., Figs. 5 and 6.

It can readily be seen that in Fig. 6 if we take the reaction rate to be $\left(\frac{de}{dt}\right)$ we would be very much in error. However, in Fig. 7 there is little difference. Therefore, the stirring must be vigorous. The correction in Fig. 6 is very much larger than the measured evolution, and thus any error in $\left(\frac{de}{dt}\right)_c$ leads to vast changes in V_c. This is not so for Fig. 7, thus if possible the reaction should be studied with rapid stirring rather than corrected slow stirring.

$$\begin{aligned} \mathcal{V}_c &= e_c + V_c \\ &= e_c + fn\left(\frac{de}{dt}\right)_c \\ e_c &= \mathcal{V}_c - fn\left(\frac{de}{dt}\right)_c \end{aligned}$$

For a given reaction at time t_c, $\mathcal{V}_c$ is a constant. In the case of slow stirring $\mathcal{V}_c$ is nearly equal to $fn\left(\frac{de}{dt}\right)_c$ and thus a slight change in the latter due to change in stirring rate, will cause their difference to vary considerably, i.e., e_c will depend on stirring rate. At the faster stirring rate $fn\left(\frac{de}{dt}\right)_c$ is a small percentage of $\mathcal{V}_c$ and their difference is almost ndependent of any change in $\left(\frac{de}{dt}\right)_c$, i.e., e_c will be independent of stirring rate. This is as has been reported and justifies the usual test for efficient stirring, namely, if a reaction rate, i.e., $\left(\frac{de}{dt}\right)$ does not alter with more rapid stirring, the measured reaction rate is equal to the actual reaction rate.[13] That is, the correction for supersaturation is negligible.

For the above data and apparatus the measuring system can be relied upon as accurate to within 0·01 c.c. and if we require the system to be independent of stirring rate then the volume stored in solution must be less than 0·01 c.c., i.e., at this value for V for rate

$$(a)\ \left(\frac{de}{dt}\right)_c = 0{\cdot}0005 \text{ c.c./min.}$$

$$(b)\ \left(\frac{de}{dt}\right)_c = 0{\cdot}0060 \text{ c.c./min.}$$

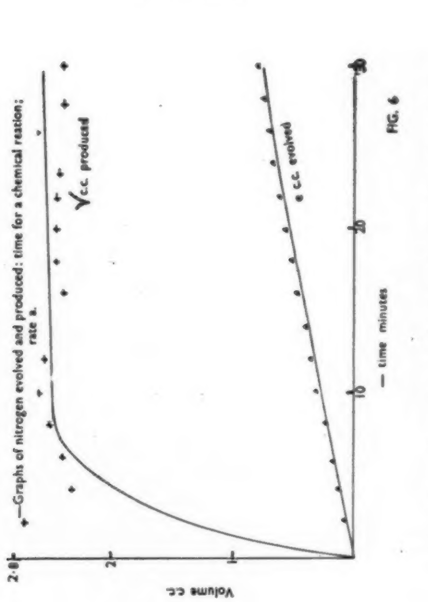


FIG. 6—Graphs of nitrogen evolved and produced: time for a chemical reation; rate a.

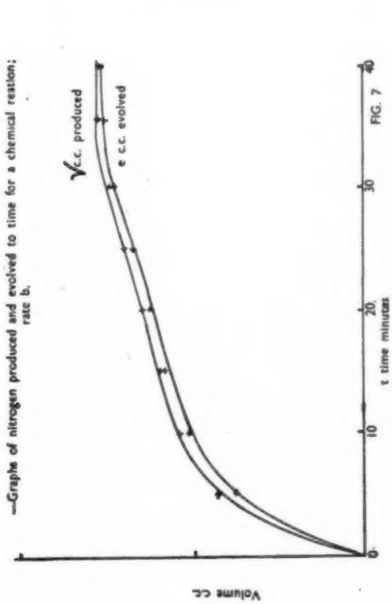


FIG. 7—Graphs of nitrogen produced and evolved to time for a chemical reation; rate b.

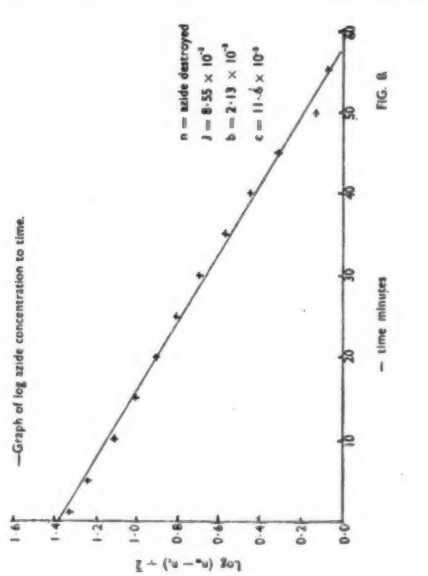


FIG. 8—Graph of log azide concentration to time.

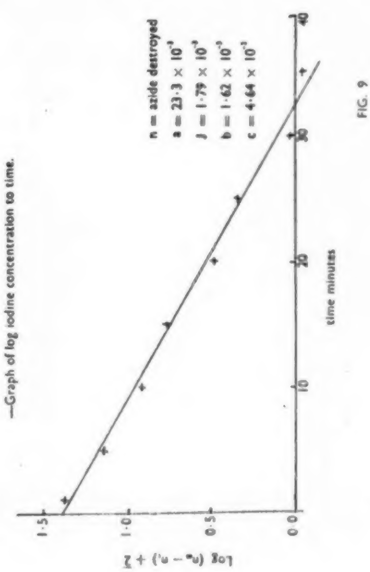


FIG. 9—Graph of log iodine concentration to time.

Under these conditions e is always within 0·01 c.c. of V and the rate of reaction is within the accuracy of the measurements equal to the rate of evolution and independent of stirring rate provided it remains less than the above values. It is to be noted that if the reaction rate is very rapid, stirring cannot be made efficient enough and a study by gas evolution will be useless; for a slow reaction very rapid stirring is not necessary for accuracy.

Note: 1. Portion c.d. of Fig. 4 is a straight line

$$\left(\frac{de}{dt}\right)_c = 0{\cdot}00515 + 0{\cdot}0102\ V_c$$

$$\hat{6}\left(\frac{de}{dt}\right)_c = 0{\cdot}0012$$

as fitted by least squares regarding V as accurate compared with $\left(\frac{de}{dt}\right)_c$

2. Fig. 5 is a straight line

$$\left(\frac{de}{dt}\right)_c = 0{\cdot}628\ V_c$$

$$\hat{6}\left(\frac{de}{dt}\right)_c = 0{\cdot}088$$

Kinetic study of reactions involved

The chemical reactions taking place are those between iodine and sodium azide and between sodium sulphide and iodine.

$$I_2 + 2\bar{N_3} \xrightarrow[K_1]{S^{--}} 3N_2 + 2\bar{I} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$S^{--} + I_2 \underset{b\,K_3}{\overset{K_2\,a}{\rightleftharpoons}} S^{\circ} + 2I^{-} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Reaction 1 is catalysed by sulphide ion and is irreversible. Reaction 2 is reversible[14, 15] but, it is not a homogenious reaction since sulphur is insoluble in aqueous solution. Reation

$$S^{\circ} + 2I^{--} \xrightarrow{K_3} S^{--} + 2I \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2a)$$

is in effect a physical solution of sulphur. The sulphur is formed as a colloid and if the iodide concentration is raised so as to produce a measurable rate the colloid is precipitated.

The reaction does not lend itself to easy study. However, it can be demonstrated by the production of iodine and hydrogen sulphide when sulphur is kept in a saturated solution of potassium iodide for some hours.

$$\text{Reaction } S^{--} + I_2 \xrightarrow{K_2} S^{\circ} + 2I^{-} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2b)$$

is extremely rapid as is witnessed by the immediate precipitation of sulphur on the addition of iodine to hydrogen sulphide solution.

The following kinetic investigations were carried out using apparatus and experimental procedures similar to those for the final experiments.

At equilibrium (of reactions 2a and 2b) reaction 1 can be studied.

Dependence of reaction 1 on:

Azide concentration

Iodine was added to a solution of sodium sulphide so as to have a large excess of iodine at equilibrium, and then small amounts of sodium azide added. The reaction rate was studied by following the nitrogen evolution. Fig. 9 and 8 are graphs of the results. The reaction rate is proportional to the azide concentration.

Iodine concentration

Varying amounts of iodine were added to a fixed quantity of sulphide so as to yield varying concentrations of iodine at equilibrium. The total iodide concentration at equilibrium was kept constant. Excess azide was then added and reaction then studied. Fig. 10 and 11. The reaction rate is proportional to the iodine concentration. This is based on the assumption that reaction rate 2 b is very slow, which seems reasonable since the results show no effect due to any such possible reaction rate. The curve in Fig. 10 flattens out almost completely.

—Graph of reaction rate at equilibrium of reaction 2 to iodine at equilibrium

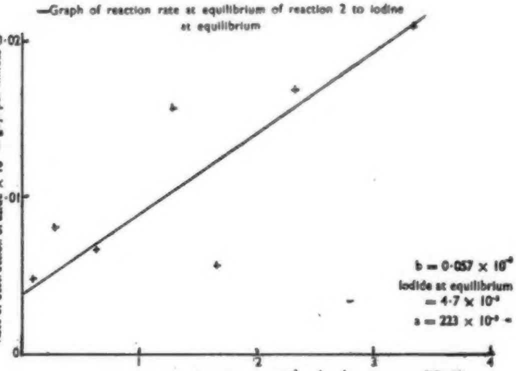


FIG. 10

—Graph of reaction extent to azide concentration.

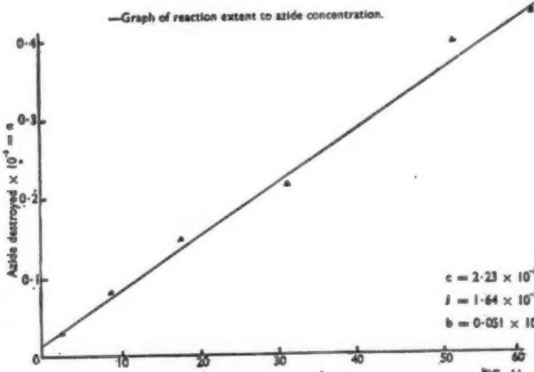


FIG. 11

Equilibrium sulphide concentration

Reaction 1 also depends on the catalyst concentration which is governed by the equilibrium position of the reactions 2a and 2b. An attempt was made to study this effect by varying the initial iodine and sulphide concentrations before addition of excess azide. The effect of iodine concentration was studied as previously only having a larger excess of iodine at equilibrium and keeping all other concentrations constant. Fig. 12 shows that rate of reaction 1 is directly proportional to iodine concentration, and since reaction 1 is proportional to iodine concentration, the catalyst concentration at equilibrium must be independent of iodine.

Fig. 13 shows the effect of iodide concentration. It is a complex effect since it is known to retard oxidation by iodine and also should effect the equilibrium position of 2. Its effect also seems to depend on the azide concentration as will be shown later.

The influence of initial sulphide concentration has been difficult to follow owing to unexpected and uncontrollable scatter of the plotted points, such that it is difficult to draw any conclusions. However, reaction 1 seems to be more rapid with increase of initial sulphide concentration, the same equilibrium concentrations of azide, iodine and iodide being maintained.

Study of competing reactions

Experiments were carried out involving the addition of iodine from the side arm to the reactants in the flask (sulphide, azide and iodide). Reaction rates could not be found since the reactions are too rapid to be studied by a gas evolution technique. However, a study of the extent of overall reaction is possible with the following results.

Effect on reaction of:

Azide concentration

Fig. 14 shows that reaction extent is proportional to the azide concentration.

Sulphide concentration

Fig. 15 shows that the reaction extent is proportional to sulphide concentration.

Iodine concentration

If a straight line is fitted to the results as shown in Fig. 16 by a least squares method and we regard the iodine values as accurate compared with the nitrogen values, we obtain

$$n = 0{\cdot}247 \times 10^{-3} - 0{\cdot}00415j$$

$$\hat{6}n = 0{\cdot}038 \times 10^{-3}$$

$$\text{whence } \hat{6} \text{ shape} = 0{\cdot}00279$$

and applying the Student's "t" test to see if the slope is significantly different from zero, that is, if reaction extent depends on iodine concentration, we obtain

$$\frac{0 + 0{\cdot}00415}{0{\cdot}00279} = 1{\cdot}48 \text{ and t for 8 degrees of freedom is } 1{\cdot}86$$

at 90 per cent level. Iodine dependence is not significant at 90 per cent. The slope anyway is very slight.

Effect of iodide concentration

The iodide concentration would appear from Fig. 17 to markedly effect the extent of reaction at the given azide concentration. However, when experiments were conducted at high azide concentration (cf. Fig. 15), the iodide concentration could be varied from the value given to 124×10^{-3}N. without any change in extent of reaction. It

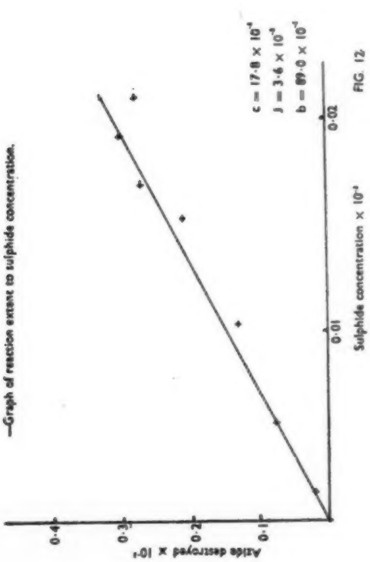


FIG. 12.—Graph of reaction extent to sulphide concentration.

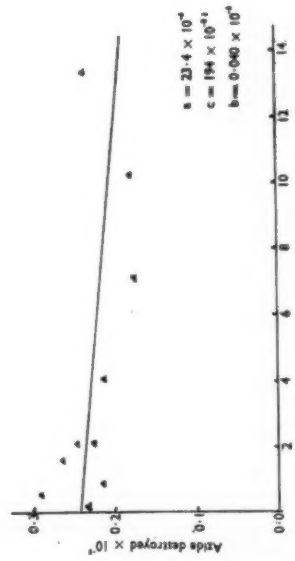


FIG. 13.—Graph of reaction extent to iodine concentration.

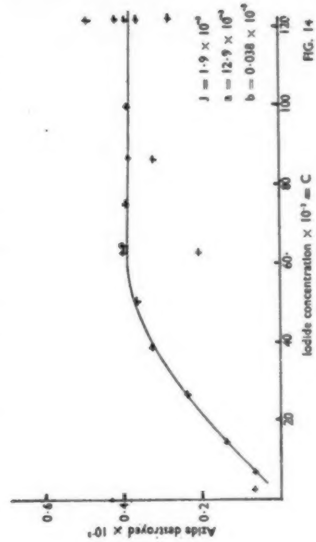


FIG. 14.—Graph of reaction extent to iodide concentration.

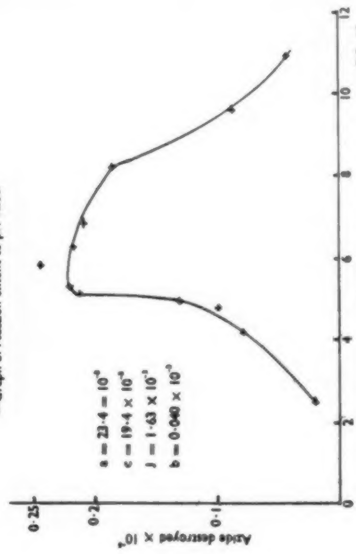


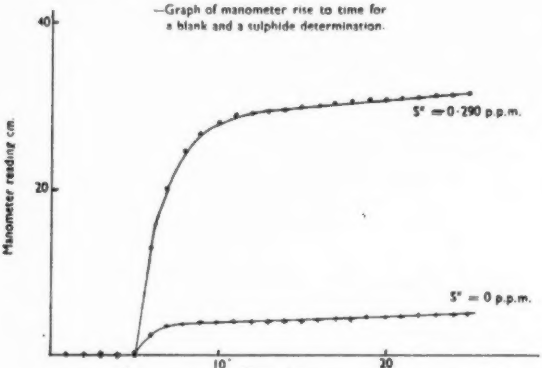
FIG. 15.—Graph of reaction extent to pH value.

would appear that the effect of iodide concentration depends on the azide concentration.

It is also to be noted that at higher iodide values the scatter of the plotted points increases. This makes it difficult to investigate both the effect of the iodide and the iodine, since high concentrations of the latter are not obtainable without similar concentrations of the former (e.g., Fig. 16).

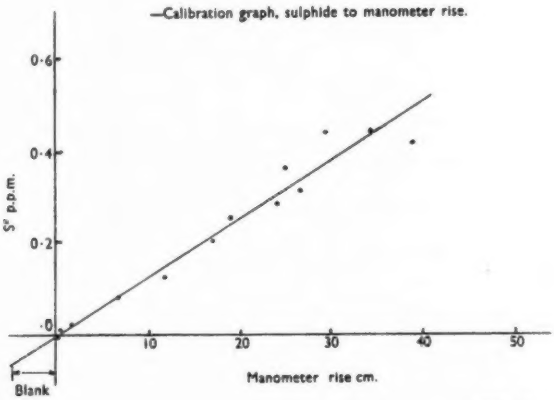
Effect of pH value

As is shown in Fig. 18 it has been found that the reactions are influenced by the pH value of the reaction mixture; the most suitable pH is between pH 5·1 and pH 7. This is the range in which the kinetic studies were carried out. The buffer system used was the azide-hydrazoic acid system when there was a high enough azide concentration and the phosphoric acid—sodium phosphate system in all other cases. The acetic acid—acetate system was found to cause variable effects, probably due to the increased solubility of sulphur in acetate solution.



—Graph of manometer rise to time for a blank and a sulphide determination.

FIG. 16



—Calibration graph, sulphide to manometer rise.

FIG. 17

DISCUSSION

From the investigation on reaction 1 it is apparent that the reaction can be written as follows:—

$$dn/dt = K_1 (a-n) (b-s) (j-n-s)$$

where n = normality, azide destroyed,
b = normality, initial concentration of sulphide,
a = normality, initial concentration of azide,
s = normality, sulphide changed to sulphur,
j = normality, initial concentration of iodine.

For rate of reation 2, we can write.

$$ds/dt = K_2 (b-s) (j-n-s) - fn\,(s.c+s+n).$$

Where s = concentration of iodide, normality

$$\text{i.e., } dn/ds = \frac{K_1 (a-n)(b-s)(j-n-s)}{K_2 (b-s)(j-n-s) - fn.\,(s.c+s+n).}$$

To solve this equation, firstly assum that the reverse reaction 2b is negligible and that azide concentration is maintained high enough to be considered invarient as is the case in the data reported.

Hence

$$dn/ds = \frac{K_1\, a\, (b-s)\, (j-n-s)}{K_2\, (b-s)\, (j-n-s)}$$

$$\text{i.e., } n = \frac{K_1\, a\, s}{K_2} + C$$

$$\text{when } n = o,\ s = o\ \therefore\ C = o$$

$$\text{i.e., } n = \frac{K_1\, a\, s}{K_2}$$

Thus the reaction extent is proportional to azide and sulphide concentration (cf. Fig. 14 and 15), and independent of iodine concentration, which seems probable from Fig. 16 and discussion thereof. It must be remembered, however, that the above is only an approximation to the truth, since we have neglected the reversibility term from equation 2b. This thus neglects the effect of iodide.

It has been suggested that the mechanism of the above series of reactions entails the liberation of atomic iodine[16]. In a manner analagous to the thiosulphate catalysis. This is possible, but seems doubtful due to a definite catalytic effect at equilibrium of reactions 2. A mechanism on the same lines as the cystine catalysis[9] is more probable. From Fig. 14 and 15 we can obtain the ratio of the reaction constants. K_1/K_2, these give $0{\cdot}132$ and $0{\cdot}145 \times 10^{-3}$ lites/g. equiv. respectively.

CONCLUSION

The reaction

The preceding discussion indicates:—

1. A high relative azide concentration is required to increase the sensitivity of the method and to minimize the effect of iodide.

2. The pH value of the reaction mixture should be about 6. This fixes the quantity of hydrochloric acid to be added.

3. Since the reaction is independent of iodine concentration sufficient must be added to allow the reaction to proceed to completion.

Procedure

Reagents required

Sodium azide solution 2·50 N.

Iodine solution 0·20 N. in 1·0 N. potassium iodide.

Hydrochloric acid solution 0·17 N.

Sample of sulphide free water.

10 ml. of sodium azide solution, 5 ml. of hydrochloric acid solution, 5 ml. of iodide solution, are pipetted into the side arm of the flask, and the whole tilted to mix the above. Into the flask 250 ml. of sulphide free water is poured and the stirrer started. The whole, after being connected to the manometer, is placed in the thermostat for 45 minutes to attain temperature and barometric equilibrium. The manometers of the reaction flask and thermobarometer are then levelled to the zero mark and the taps closed. After three minutes, readings are taken, each minute for three minutes, and then the side-arm reactants run into the flask. After 17 minutes, the manometers are read each minute for three minutes and the mean of the first three subtracted from the mean of the latter three, all being corrected for any thermobarometric change. This is taken as the manometer rise. The above is repeated for the unknown sample of water. A typical determination is shown in Fig. 19. The difference between the reading for the sample and the blank is read from the calibration graph as sulphide in p.p.m.

Calibration

A freshly prepared 0·02N. sodium sulphide solution is standardized with iodine, immediately after standardization a solution of required sulphide contents is prepared by dilution and the manometer rise found as previously. From a series of such determinations a calibration graph (manometer rise against sulphide concentration) is plotted.

A typical graph is shown in Fig. 20. A least squares line $y = px + q$ has been fitted to the points assuming (1) that the scatter of the points obeys the normal law, (2) that the standard deviation at any manometer reading is proportional to that value $\delta y = x\delta$ (x = manometer rise + blank), (3) that the manometer rise is more accurately de termined than the sulphide concentration. This last assumption seems justified since dilute solutions of sulphide deteriorate rapidly, and when using the method for a determination of an unknown, the manometer rise is the value read and must be assumed accurate. For the apparatus

$$S^{--} = 0{\cdot}0127x - 0{\cdot}0567 \text{ p.p.m.}$$

Thus sulphide can be determined over the range 0·5 p.p.m. to 0·01 p.p.m. with a standard deviation of $x\delta = 0{\cdot}0527$ at the high value and at 0·01, $x\delta = 0{\cdot}006$, i.e., limit sensitivity approximately 0·010 p.p.m.

The authors thank Professor H. Stephen for his interest and encouragement, the South African Council for Scientific and Industrial Research for a grant to one of them (R. E. P.) and African Explosives and Chemical Industries Limited, for supplying the sodium azide used in the investigation.

Department of Chemistry and Chemical Engineering,
University of the Witwatersrand.
Johannesburg.

Received February 23, 1952

REFERENCES

[1] Pomeroy, R., *J. Amer. Water Works Assoc.*, 1941, **33**, 943.
[2] Fogo, J. K. and Popowsky, M., *Anal. Chem.*, 1949, **21**, 732.
[3] Goto, H. and Takanolu, S., *J. Chem. Soc. Japan*, 1944, **65**, 673.
[4] Feigl, F., *Z. Anal. Chem.*, 1928, **74**, 369.
[5] Metz, L., ibid, 1929, **76**, 347.
[6] Feigl, F., *Microchemie N. F.* 1934, **9**, 1.
[7] Raschig, F., Ber., 1915, **48**, 2088
[8] Friedmann, *J. Prak. Chemie. N.F.*, 1936, **146**, 179.
[9] and [10] Compt. Rend. Trav. Lab. Carlsberg, Ser Chim., 1949, **27**, 72, 63.
[11] Dixon, M., "Manometric methods", Cambridge University Press, 1934, p.31.
[12] Everard-Lamplough, F. E., Proc. Camb. Phil. Soc., 1906–1908, **14**, 580.
[13] England, W. B. and Mackenzie, H. A. E., *J. S. A. Chem. Inst.*, 1949, **2**, 147.

A SENSITIVE ELECTROMETRIC METHOD FOR THE DETERMINATION OF SULPHIDE ION IN SOLUTION

by

R. E. PRESS and K. A. MURRAY

OPSOMMING

Jodium wat elektries vrygestel is, word gebruik om onder katalitiese invloed van die sulfied-ioon, die asied-ioon te titreer. Die endpunt word bereik wanneer die katalis deur die jodium vernietig is, waarna die oormaat jodium amperometries bepaal word. Sulfiedkonsentrasies van 0.08 tot 0.01 d.p.m., kan in 'n 75 ml. monster bepaal word, met standaardafwyking van 0.0032 d.p.m., en konsentrasies so laag as 0.0025 d.p.m. kan met 95 persent sekerheid bepaal word.

SUMMARY

Electrically generated iodine is used to titrate the azide ion under the catalytic influence of the sulphide ion. The end point is reached when the catalyst has been destroyed by the iodine and the excess iodine thereafter is detected amperometrically. Sulphide can be determined in the range 0.08 to 0.01 p.p.m. on a 75 ml. sample with a standard deviation of 0.0032 p.p.m., and detected down to 0.0025 p.p.m. with a certainty of 95 per cent.

INTRODUCTION

The sulphide ion can be determined directly by titration with electrically generated iodine under conditions similar to those reported by Ramsey Farrington and Swift[1] for the titration of arsenious acid. This involves the generation of iodine in the solution by one pair of platinum electrodes supplied with a constant current of the order of 1–10 milliamps, and determination of excess iodine by a second pair of platinum electrodes, and plotting diffusion current to time curves. The use of the amperometric endpoint was found to give a very sensitive indication of excess iodine. It was decided that if azide ion were present in the titration system the iodine required to oxidize the sulphide would be increased because of the side reaction between iodine and azide which is catalysed by the sulphide ion.[2]

Apparatus

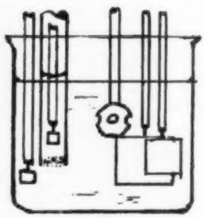
The apparatus is similar to that of Swift except that a larger sample was taken. The electrodes dip into a 100 ml. beaker stirred by a stirrer of the type shown in fig. 1 working at 2,800 r.p.m. This was found to be more suitable than a propeller type since it avoided entrainment and provided very efficient and smooth stirring. The larger sample volume makes timing much simpler since the slope of the diffusion current to time curve at the end point is less steep and loss of accuracy due to this is compensated for by the longer generation time required.

The generating current, which could be kept constant to within 0·5 per cent, was supplied from a 45-volt bank of Edison cells. As the more significant error in the titration is due to the instability of the dilute sulphide samples it was considered unnecessary to use an electronic generator source. The high generation rate—used only to sensitize the electrodes—was 10 milliamps, and the low generation rate was 1·019 milliamps. A polarizing potential of 1·5 volts was applied to the indicator electrodes.

Determination

The experimental technique is as used by Swift with the following modifications: (1) a sample of 70 ml. is used, to which is added 5 ml. 1N–KI, 5 ml. 1N–NaN_3, and 1 ml. 0·17 N–HCl solutions (pH 6·0); (2) The cathode shield is filled with a solution of

Fig. 1



Apparatus

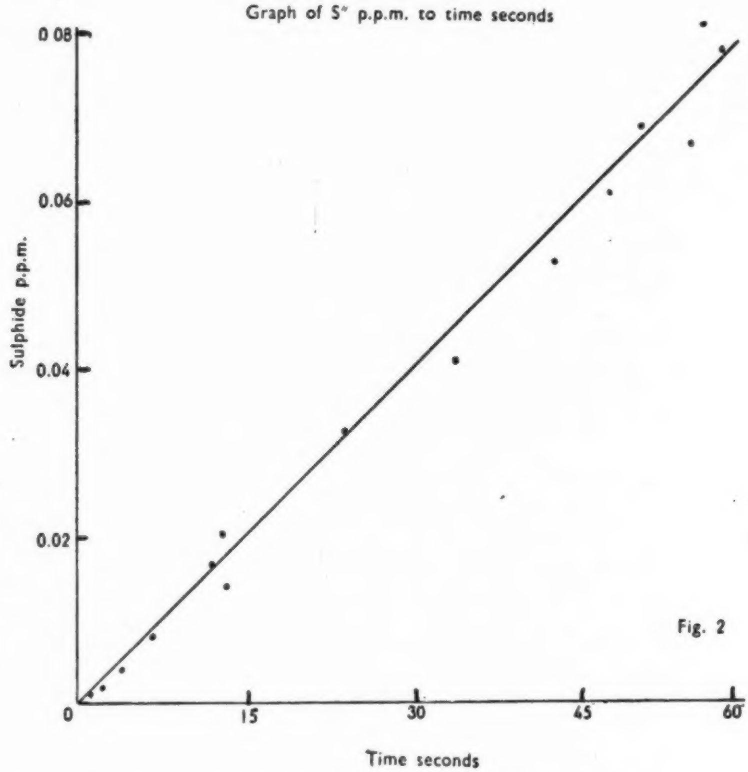


Fig. 2

the same composition as the above, using 70 ml. sulphide free water; (3) In view of the larger sample volume the generator current is on for 5-second intervals during the endpoint determination; (4) The indicator current is read 5 seconds after stoppage of genration and generation immediately restarted. This is due to the downward drift of the indicator current. The temperature of the sample should be 20 ± 2°C.

Using the above concentrations of reactants the following equation gives the sulphide concentration at generation rate r milliamps, which should be approximately 1 milliamp, in terms of the time t in seconds.

$$S^{--} \text{ p.p.m.} = 0 \cdot 00126tr.$$

Calibration

The above equation is obtained from the following calibration. A freshly prepared 0·01 M. sodium sulphide solution was standardized with iodine. Immediately after standardization a solution of required sulphide content was prepared by dilution with water free from sulphide and oxidizing agents, and titrated according to the above procedure. Figure 2 shows the plotted results of such a series.

The graph is a straight line passing through the origin with slope = 0·00128 as fitted by least squares assuming the time to be accurately known as compared with the sulphide value. The standard deviation is 0·0032 p.p.m.

From a series of ten blank determinations $\hat{\sigma}_t$ = 0·85 seconds and thus 0·0025 p.p.m. can be detected by three determinations on the sample and three blanks (time one hour) with a degree of certainty of 95 per cent, as given by a Students t test. On ten determinations on each the sensitivity is increased down to 0·0005 p.p.m. However, such a value would have little significance due to the presence and interference of trace impurities.

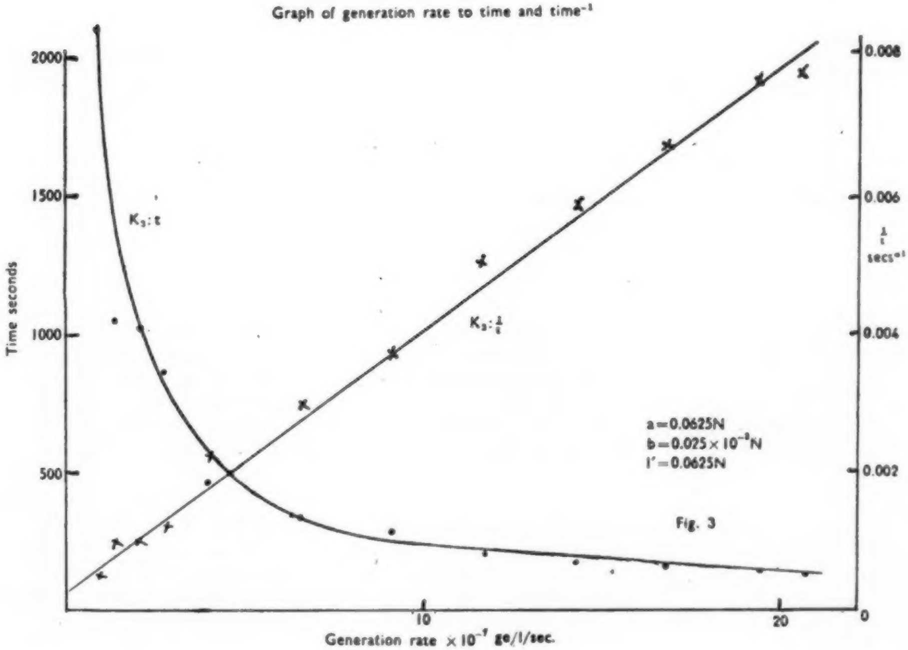
DISCUSSION

It was found that the presence of azide has little effect on the diffusion current sensitivity and a polarizing potential of 1·5 volts was found suitable.

The pH value suitable for the catalysis reaction is 6·0 and this is also suitable for the iodine generation and indication. The buffer used is the azide hydrazoic acid system.

As the reactions involved are not direct oxidation but also catalysis, the effect of change of reactant concentration was investigated. Series of runs were made at a pH of 6·0 keeping all reactants and conditions the same except the reactant under investigation. The effect of change of generation rate was also investigated. c.f. Figs. 3, 4, 5 and 6.

Graph of generation rate to time and time^{-1}



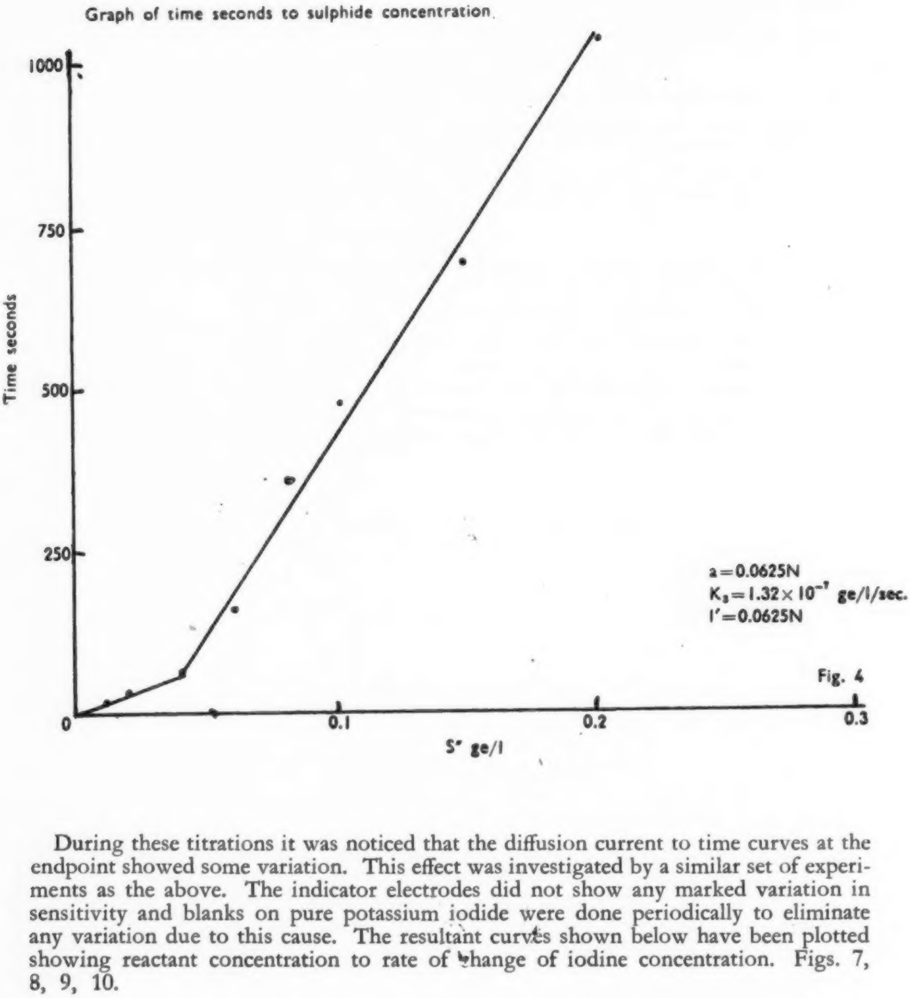


Fig. 4

During these titrations it was noticed that the diffusion current to time curves at the endpoint showed some variation. This effect was investigated by a similar set of experiments as the above. The indicator electrodes did not show any marked variation in sensitivity and blanks on pure potassium iodide were done periodically to eliminate any variation due to this cause. The resultant curves shown below have been plotted showing reactant concentration to rate of change of iodine concentration. Figs. 7, 8, 9, 10.

Temperature does not markedly affect the time as recorded in Figs. 3 to 6, but does affect the rate of increase of diffusion current to time curves at the endpoint. This latter is due mainly to the characteristics of the diffusion mechanism. The results in Table I show that if the sample is kept at $20 \pm 2°$ C. there is no need for thermostating. The standard deviation as reported under "calibration" is for an unthermostated sample.

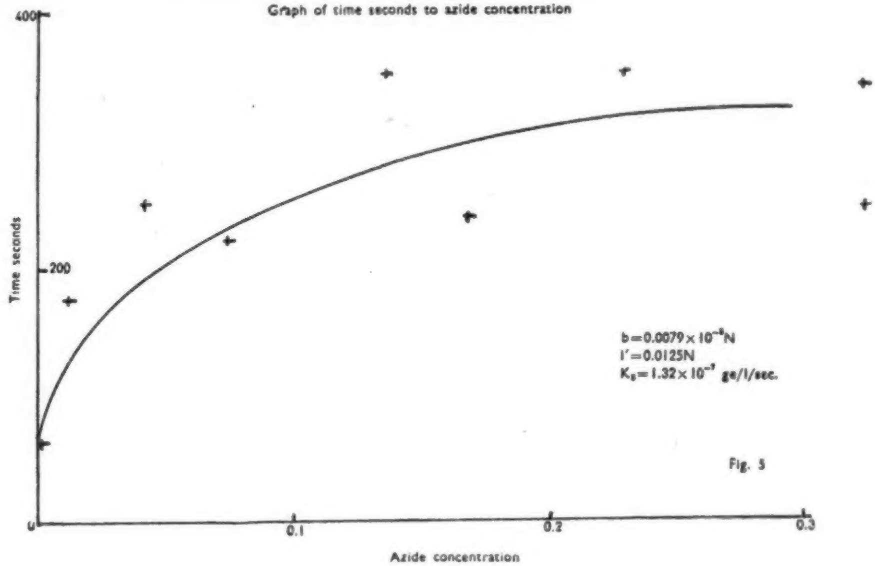

Graph of time seconds to azide concentration
Time seconds
400
200
0
0.1
0.2
0.3
Azide concentration
b=0.0079×10⁻³N
I′=0.0125N
K₂=1.32×10⁻⁷ ge/l/sec.

Fig. 5

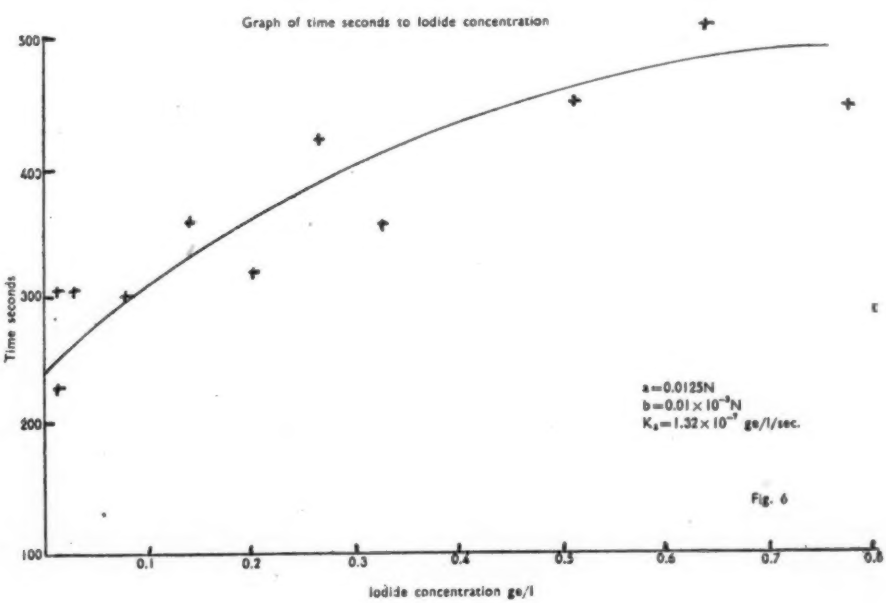

Graph of time seconds to Iodide concentration
Time seconds
500
400
300
200
100
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Iodide concentration ge/l
a=0.0125N
b=0.01×10⁻³N
K₂=1.32×10⁻⁷ ge/l/sec.

Fig. 6

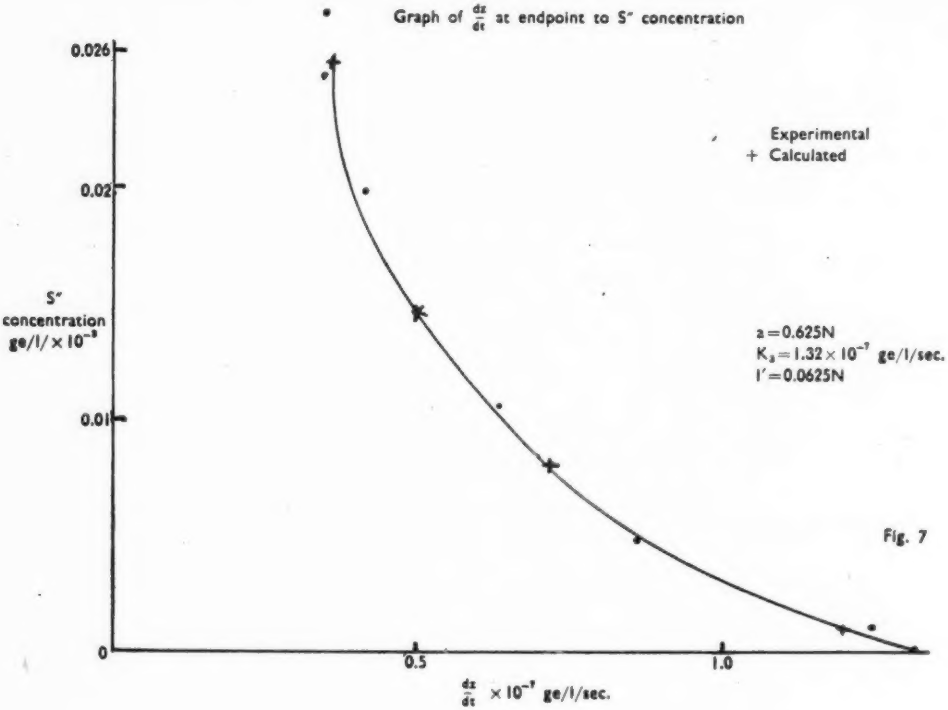


Fig. 7

TABLE I

Temperature ° C.	*Time secs.*	*Slope of diffusion current to time curve*
10	35	3·6
24	38	5·9
40	32	6·1
67	27	7·1

DISCUSSION

The mechanism by which the reactions take place is that the iodine, generated at a constant rate, will maintain a concentration such that the rate of increase of iodine concentration is equal to the rate of production minus the rate of the reactions.

Graph of $\frac{dz}{dt}$ at endpoint to azide concentration

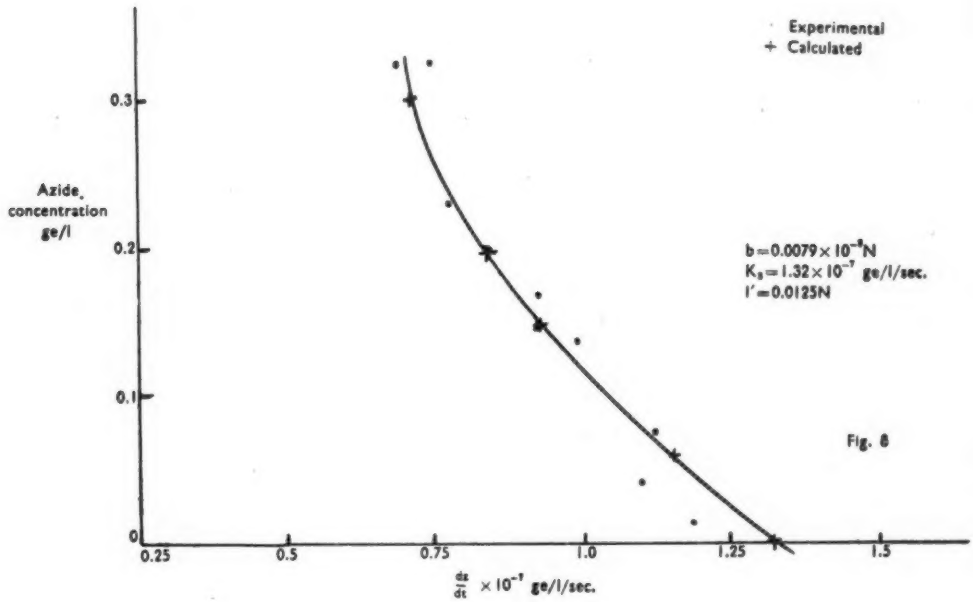


Fig. 8

The reactions are:

$$2N_3^- + I_2 \xrightarrow[S^{--}]{K_1} 3N_2 + 2I^- \qquad \text{(1)}$$

$$S^{--} + I_2 \underset{bK_4}{\overset{aK_2}{\rightleftharpoons}} S^\circ + 2I^- \qquad \text{(2)}$$

The rate of these reactions can be written thus:—

$$dn/dt = K_1\, a(b-s)\,(j-n-s) \qquad \text{(3)}$$

$$ds/dt = K_2\,(b-s)\,(j-n-s) \qquad \text{(4)}$$

neglecting reaction 2b and assuming no significant change in azide concentration. The reactions here taking place can be written:—

$$dz/dt = K_3 - z(b-s)aK_1 - z(b-s)K_2 \qquad \text{(5)}$$

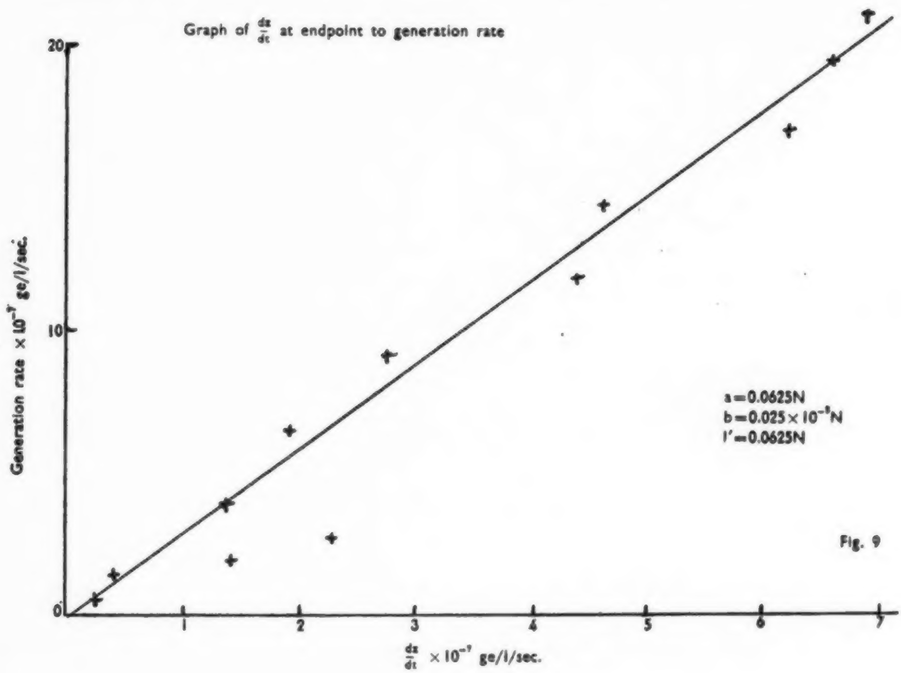


Fig. 9

Where in terms of normality, n = azide destroyed in time t,
s = sulphide oxidized in time t,
and b = sulphide at time t = o,
a = azide at time t = o

j–n–s = z is concentration of iodine at time t. K_3 is the rate of generation of iodine, K_1 is velocity constant reaction 1, K_2 velocity constant reaction 2a.

From the experimental results it would appear that z remains small for some time and then rises linearly, the time as recorded in Figs. 3, 4, 5 and 6 is this period of low iodine concentration and the sensitivity in Figs. 7, 8, 9 and 10 is the rate of linear rise thereafter. A full discussion therefore requires an equation for iodine concentration to time. This requires a solution of equations 4 and 5, which is difficult without doubtful approximations. However, if it is assumed that $dz/dt = 0$ in equation 5, which it approximately is up to the end point as taken, then:—

$$ds/dt = K_2K_3/(K_1a + K_2)$$
$$\text{i.e. } t = (K_1a + K_2)b/K_2K_3$$

Thus t should be proportional to $1/K_3$, s, and a + const. These results do not conform in all cases to the curves as shown in Figs. 3, 4 and 5. The values of K_1/K_2 as calculated from the slope of curves 3 and 4 are $0{\cdot}147 \times 10^3$ and $0{\cdot}106 \times 10^3$ litres/g.equiv., these values are close to those obtained by a manometric method.[2]

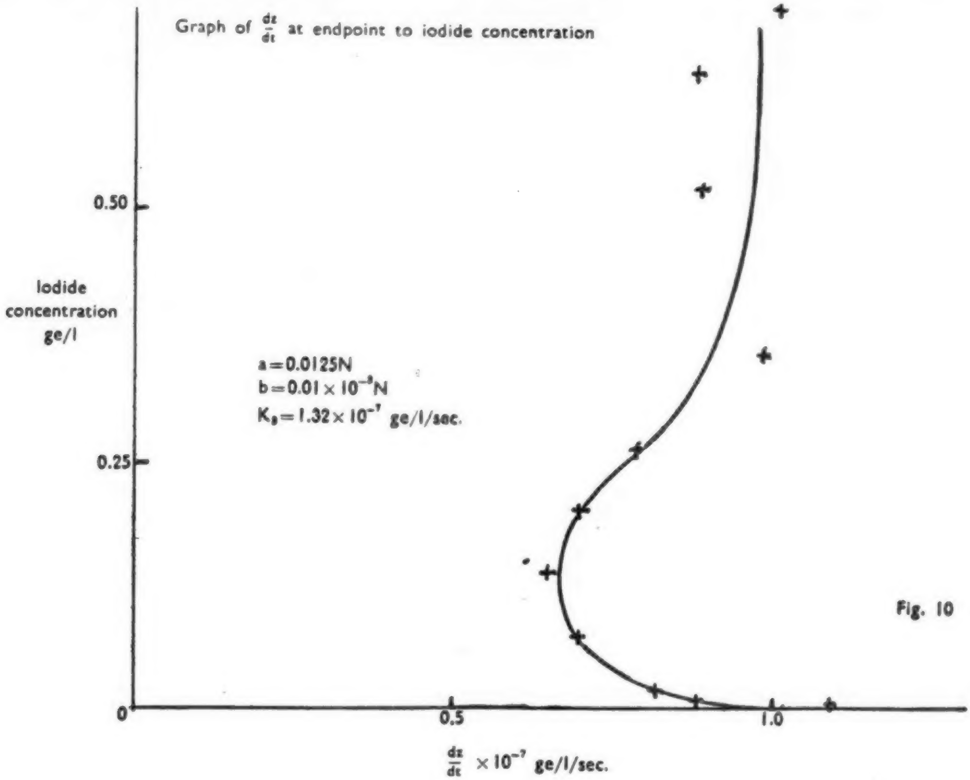


Fig. 10

A large portion of the reactions takes place in the diffusion layer surrounding the generation electrode, in which region the above equations will not apply. Since azide is the only important rate-determining reactant in large concentration, its effect will be most influenced by the above diffusion layer. This may account for the discrepency of the experimental effect of azide.

The endpoint sensitivity, as taken, is influenced by the reactant concentrations since the rate of increase of iodine concentration is the difference between the generation rate and the reaction rates 1 and 2a. The latter cannot become zero since reaction 2 is reversible and thus the rate of increase of iodine at the endpoint is always less than the generation rate, provided some sulphide and azide are present. The following empirical equation is obtained from results of experiments Figures 7 and 8, and the results as calculated from it shown plotted:

$$dz/dt = K_3c/(ab+c); \quad c = 0{\cdot}58\times10^{-6},\ I^- = 0{\cdot}0625N. \text{ Fig. 7.}$$
$$c = 2{\cdot}77\times10^{-6},\ I^- = 0{\cdot}0125N. \text{ Fig. 8.}$$

The authors thank Professor H. Stephen for his interest and encouragement, the South African Council for Scientific and Industrial Research for a grant to one of them (R.E.P.) and to African Explosives and Chemical Industries, Limited, for supplying the sodium azide used in the investigation.

Department of Chemistry and Chemical Engineering,
University of the Witwatersrand,
Johannesburg.

Received April 8, 1952.

REFERENCES

[1] Ramsey, W. J., Farrington, P. S., Swift, E. H., *Anal. Chem.*, 1950, **22**, 332.
[2] Press, R. E. and Murray, K. A. This journal.

COUNCIL, 1st JULY, 1951—30th JUNE, 1952
RAAD, 1 JULIE, 1951—30 JUNIE, 1952

President:
L. F. Addis-Smith, M.Sc., A.R.C.S., D.I.C., F.R.I.C.

Immediate Past President/Vorige-president:
P. R. van der Riet Copeman, B.A., B.Sc., D.Sc.

Vice President/Vise-president:
A. J. Petrick, M.Sc., Dipl. Ing., Dr. Ing.

Hon. Secretary and Treasurer/Ere-sekretaris en Tesourier:
C. F. Lindemann, B.Sc.

Hon. Editor and Librarian/Ere-redakteur en Bibliotekaris:
K. A. Murray, M.Sc., Ph.D., F.R.I.C.

Members of Council/Raadslede:
S. Transvaal Section/Seksie:
F. Hawke, M.Sc.(Eng.), Ph.D., F.R.I.C.;
F. W. Hayes, F.R.I.C.;
S. S. Israelstam, M.Sc., Ph.D., F.R.I.C.;
E. G. White, M.Sc., F.R.I.C.

Natal Section/Seksie:
W. H. Dyson, M.A., F.R.I.C.;
J. L. du Toit, M.Sc.;
L. B. Petters, B.Sc., A.M.I.Chem.E.

Pretoria Section/Seksie:
A. W. Lategan, M.Sc., Ph.D.;
J. G. Louw, D.Sc.;
G. W. Perold, D.Sc., Dr.sc.techn.

E. Province Section/O. Provinsie Seksie:
R. Schauder, B.Sc.;
E. P. Stevens, Ph.D., B.Pharm., Ph.C., A.R.I.C.

W. Province Section/W. Provinsie Seksie:
A. L. Abbott, B.Sc., F.R.I.C.;
H. W. Schirach, B.Sc., A.R.I.C.;
E. N. Serebro, B.A., A.R.I.C.

VIR DIE AANDAG VAN OUTEURS

Die Joernaal van die Suid-Afrikaanse Chemiese Instituut word gewy aan die publikasie van oorspronklike navorsing in alle vertakkings van chemie en chemiese tegnologie.

Alle bydraes moet in triplikaat ingehandig word; indien daar grafieke is moet een stel noukeurig met *Oosindiese ink afgeteken word op gladde wit Bristol-blad of tekenpapier, of op aftrekdoek*—die ander twee stelle kan afdrukke wees, of met potlood geteken word.

Artikels moet so saaklik moontlik wees, verenigbaar met duidelikheid, en moet nie onnodige eksperimentele besonderhede bevat nie. Artikels moet *in tikskrif wees—dubbel spasiëring*—net aan een kant van die papier, met 'n behoorlike kantruimte aan die linkerkant. Die papier moet nie-absorberend wees.

Die buitemate van plate—insluitende die ruimte vir byskrif—moet hoogstens 14 duim by 10 duim wees, en liefs nie minder as 7 duim by 5 duim nie. Die verhouding 1.4 : 1.0 moet in elk geval nagekom word. Byskrif en letters—hetsy in die kantruimte of in die tekening self—moet nie met ink gedoen word nie, *maar liggies met gewone potlood of uitveebare bloupotlood.*

Elke artikel moet voorafgegaan word deur 'n opsomming in elk van die twee offisiele landstale, en die opsomming wat tweede verskyn moet in dieselfde taal as die artikel wees. Die opsomming moet in eenvoudige taal 'n kort en saaklike uiteensetting gee van die oogmerke van die ondersoek, die resultate wat behaal is, en die gevolgtrekkings. Die opsomming moet ongeveer 50–250 woorde beslaan en mag in elk geval nie meer as 5 persent van die artikel wees nie.

Verwysings moet in numeriese opeenvolging aan die end verskyn, in die volgorde: skrywer se van; skrywer se voorletters; joernaal; datum, deel; bladsy; die ooreenstemmende verwysingsnommers moet in volgorde in die teks verskyn as kopskrifte.

Verwysings na boeke moet as volg aangedui word: skrywer se van; skrywer se voorletters; titel; uitgawe; uitgewer; datum van publikasie; bladsy.

Simbole en afkortings moet volgens B.S. 813 : 1938 wees.

Bydraers moet afskrifte hou van al hulle mededelings.

Outeurs word versoek om hulle bydraes so op te stel dat dit ooreenstem met die gewone trant, konvensie en kernagtigheid van hierdie joernaal.

Dui asseblief aan waarheen die drukproewe moet gestuur word.

Outeurs kan verdere voorskrifte en aanwysings, i.v.m. die opstel van 'n artikel, aanvra.

Rig asseblief alle korrespondensie aan die Ere-redakteur, Suid-Afrikaanse Chemiese Instituut, Kelvinhuis, h/v Marshall- en Hollardstraat, Johannesburg.

NOTICE TO AUTHORS

The Journal of the South African Chemical Institute is designed for the publication of original work in all branches of chemistry and chemical technology.

All papers must be submitted in triplicate: where diagrams are included one set of these must be carefully drawn, *in Indian ink, on smooth Bristol board or paper, or on tracing cloth*, the remaining two copies may be pencil drawings or prints.

Papers should be as brief as possible compatible with clarity, and no unnecessary experimental details should be included. All papers must be *typewritten in double line spacing*, on one side only of the paper, with a good margin on the left-hand side. The paper should be non-absorbent.

The total external dimensions of illustrations (including space to be taken up by lettering) should not exceed 14 inches by 10 inches and preferably should not be less than 7 inches by 5 inches—the proportion 1.4 : 1.0 being in all cases maintained. Lettering on drawings, whether on the margin or in the body of the drawing must not be in ink but must be inserted *lightly, in blue pencil or erasable lead pencil.*

Every paper must be preceded by two summaries, one in each official language; the second summary being in the same language as that of the paper. Each summary should set forth concisely and in simple language the objects and purpose of the investigation, the results obtained and conclusions drawn. These should normally be from 50 to 250 words in length and, in any case, should not exceed 5 per cent of the paper.

References should appear at the end of the paper, in numerical order and in the following sequence: author's surname; author's initials; journal; date; volume number; page; the necessary reference numbers should appear, in sequence, as superscripts in the text.

Where reference is made to books the following sequence should be adhered to: author's surname; author's initials; title; edition; publisher; date of publication; page.

Symbols and abbreviations used should conform with B.S. 813 : 1938.

Authors should retain copies of their communications.

Authors are requested to arrange their papers to conform, as far as possible, with the normal style, conventions and conciseness of this journal.

The address to which proofs are to be sent should be written on every paper.

Directions to authors regarding the preparation of papers will be forwarded on application.

All communications should be addressed to the Honorary Editor, The South African Chemical Institute, Kelvin House, cor. Marshall and Hollard Streets, Johannesburg.

ALEX WHITE & CO. (PTY.) LTD. THE WHITE HOUSE, SAUER STREET, JOHANNESBURG, SOUTH AFRICA